THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LVI

BALTIMORE, MD.
1921

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No. 1. May 1, 1921

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 56 MAY 1, 1921 No. 1

REACTIONS TO HEMORRHAGE

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Received for publication December 17, 1920

Many of the physiological reactions to hemorrhage have long been known and carefully studied. It was early recognized that after any considerable loss of blood, body fluids enter the circulation in an attempt to restore volume. With the development of blood pressure reading devices, it was found that the vasomotor center tends to bring about a general constriction of the peripheral blood vessels, which compensates for the decreased viscosity of the blood and diminishes the capacity of the circulatory system so that blood pressure may be maintained and an adequate supply of blood furnished to the central nervous system and other vital organs. The larger arteries also passively accommodate themselves to the decreased volume. Arterial blood pressure remains practically constant until blood equalling 2 to 3 per cent of the body weight is lost. The pulse rate increases with the advancing hemorrhage due most likely to decreased vagal tonus. A faster respiratory rate may facilitate venous return. Although it is obvious that sooner or later the curtailment of venous return and the interference with diastolic filling of the ventricle must decrease the cardiac output, actual experiments on the part played by the heart in the compensations to hemorrhage have been very few and the exact point at which hemorrhage decreases output has never been determined. Johansson and Tigerstedt (1), using the pericardial sac as an uncalibrated cardiometer, showed that the heart empties itself more completely after hemorrhage. Wiggers (2), (3) in excellent reviews of the literature and studies of his own on hemorrhage, deduced from pulse pressure tracings that a latent period of a few seconds to several minutes might intervene before the filling of the ventricles was affected, this being due to the luxus

supply of blood in the venous cisterns temporarily supplying the deficit. Although Wiggers (2) states that he compared pulse pressure tracings with volume outputs as determined by a cardiometer, the data were not presented and so far as we can find there are no papers in the literature of hemorrhage in which systolic output or heart size have been directly studied.

The work presented here is briefly the effect of successive small hemorrhages on the diastolic size of the heart as determined by the x-ray, and a further investigation of the problem by the more usual laboratory methods such as the cardiometer and arterial and venous manometers.

Methods. Dogs were used in all experiments. For the x-ray examinations they were morphinized and tied to an operating board. By means of a plumb bob and a marked spot on the dog between the shoulder blades the animal could be placed under the tube in exactly the previous position. This point was always further tested by seeing if the successive negatives would superimpose, using the shadows of the ribs as guide marks. In this way one could be sure that any variation in the outline of the heart was due to a change in the heart itself and not to distortion resulting from a different position of the animal. These precautions, which keep the heart in its natural place in the thorax and rigidly control the series of x-ray photographs, are believed to be of great importance in experimental work of this kind. The pictures were made during successive inspirations with two or three short flashes from a Coolidge tube, the spark gap being about $4\frac{1}{2}$ inches and the current about 35 milliamperes.

The cannula for bleeding was placed in the femoral artery, the incision being made under cocaine. Bleeding was rapid, seldom lasting over 1 minute. X-ray plates were made before, during and at intervals after the hemorrhage.

X-rays taken as described give the diastolic shadow of the heart. For comparison the heart shadows were measured with a planimeter. A variation in the silhouette area is of course taken to mean a corresponding change in heart volume. With good films the areas can be marked off with very little error. In the dog's heart, where the apex often lies well above the diaphragm in inspiration, the only part of the outline left to the experimenter's judgment is the line across the base. If this be carried across with reference to the same intercostal space the error from this source in successive films is negligible. It is possible to take a series of observations on a normal dog with variations of only about 5 per cent.

In the experiments in which cardiometer and blood pressure readings were obtained, the animals were morphinized, etherized and the chest opened under artificial respiration. The cardiometer was of the glass bulb type with a perforated rubber membrane to fit around the auriculo-ventricular groove. Care was used in its application so that the blood flow into the ventricle might not be obstructed. Systolic and diastolic pressures were obtained with maximal and minimal valves. Venous pressure was determined by inserting a sound into the vena cava through the femoral vein and balancing the pressure against a manometer filled with salt solution.

X-ray examination after hemorrhage. According to the known reactions to hemorrhage which have already been mentioned, it has been generally thought that following a rapid loss of blood there is at once, or certainly within a few seconds, a peripheral vasoconstriction and an increased heart rate. Since the blood volume is decreased these reactions would seem to necessitate a decreased cardiac output per beat. This could be brought about by diminution of systole, or as more naturally and usually supposed, by a decrease in diastolic size, that is in cardiac filling.

X-ray photographs of the heart before and after hemorrhage show, however, contrary to the opinion just expressed, that the diastolic size of the heart is maintained even after the loss of large quantities of blood. In twenty-one experiments, each with from two to six hemorrhages, the heart did not decrease more than 5 per cent, a figure we have allowed for unavoidable variations in the method, until the loss of blood on an average equaled 2 per cent of the body weight. The lowest figure was 1.2 per cent and the highest 2.9 per cent. In the dog this represents a loss of from 11 to 28 per cent of the blood volume. In table 1 may be seen a summary of nine of these experiments. The others were quite similar but have not been included for the sake of space. Figures 1 and 2 present the data of two of the experiments not included in table 1. The steady decrease in blood volume is sharply contrasted with the sudden reduction in diastolic heart size which occurs when about 20 per cent of the blood is lost, and which would seem to indicate the breakdown of some protective mechanism.

The advantage to the heart in retaining its diastolic size is evident in light of the work of Patterson, Piper and Starling (4) and Gesell (5), who have shown that, as in the case of skeletal muscle, the efficiency of cardiac contraction depends on the length of the muscle fibers.

Just how the heart after hemorrhage secures a venous return adequate and under sufficient pressure to maintain the normal distention of the ventricle is by no means a simple problem. It is not due to any change in pulse rate. It is of course well known that hemorrhage increases heart rates, but this does not occur until considerable blood is lost, a fact not always observed in the literature. In our experiments it will be seen in the curves and in table 1 that there was usually no significant change in heart rate until the sudden decrease in cardiac size occurred. To favor the heart in retaining its diastolic size the rate would have to decrease along with the loss of blood. This did not occur in our experiments, although experiments H 13 and H 27 may at first glance seem to be exceptions. These experiments show a gradual reduction of rate due to the passing off of atropine. The rates, however, came to a standstill during the last hemorrhages just at the time when changes would have been most marked if this were a mechanism enabling the heart by complete filling to retain its diastolic size.

After the loss of large amounts of blood the pulse is greatly accelerated. This acceleration often occurs with the marked decrease in diastolic size of the heart, as may be seen in figures 1 and 2. In other experiments, however, such as H 9, H 13, H 14 and H 27, there are decreases of cardiac size unassociated with any increase in pulse rate. It would seem that the sharp decrease in size of the cardiac silhouette area was associated with some phenomenon other than changes in heart rate.

It might be said that the heart retains its size after hemorrhage due to the inflow of tissue juices which at once restores the blood volume. That hemorrhage is followed by a dilution of the blood and that this may be detected even at the end of a short hemorrhage is an old observation dating from the time of Vierordt (6) or even earlier. Textbook statements often lead us to believe that in a few minutes this inflow entirely replaces the lost fluid. Such however we do not believe to be the case. The very extensive literature on this subject has few examples of significant dilutions under 20 minutes. Scott (7) for example, found in one dog that 59 minutes after bleeding 206 cc. the hemoglobin indicated return of only 23 cc. from the tissue fluid. The quickest return was 71 cc. in 20 minutes after a hemorrhage of 212 cc. These were 8 and 7 kilo dogs respectively. Richet, Brodin and Saint-Girons (8) have recently studied the effect of hemorrhage on the specific gravity of the blood. They found that after hemorrhages of 125 cc. in dogs averaging about 15 kilos the specific gravity at once fell but became stable in

TABLE 1

Showing the effect of hemorrhages of varying degrees on heart rate, blood pressure and diastolic size of the heart. The amount bled is expressed in cubic centimeters and in per cent of body weight. Corrections have not been made for the specific gravity of blood. The area of the heart is expressed in square centimeters

EXPERIMENT NUMBER	WEIGHT IN KILOS	PROCEDURE	PER CENT BODY WEIGHT	HEART RATE	BLOOD PRESSURE	DIASTOLIC SIZE
H 8	5.0	Normal		120	126	52.9
		Bled 75 cc.	1.5	120	122	50.9
		Bled 50 cc.	1.0	220	110	42.4
H 9	4.6	Normal		82	130	46.0
		Bled 75 cc.	1.6	80	134	45.1
		Bled 60 cc.	1.3	82	116	35.3
H 11	4.2	Normal		194	110	35.7
		Bled 75 cc.	1.8	194	114	34.2
		Bled 50 cc.	1.2	204	106	30.8
H 12	5.0	Normal		216	180	39.7
		Bled 160 cc.	3.2	192	168	36.8
H 13	5.5	Normal		156	170	50.8
		Bled 50 cc.	0.9	144	160	50.7
		Bled 50 cc.	0.9	132	150	50.8
		Bled 25 cc.	0.45	120	140	46.2
H 14	5.7	Normal		168	156	48.9
		Bled 50 cc.	0.09	162	160	48.3
		Bled 50 cc.		144	160	43.2
H 27	3.5	Normal		164		37.0
		Bled 23 cc.	0.6	150		37.0
		Bled 23 cc.	0.6	144		36.5
		Bled 20 cc.	0.57	144		33.5
H 37	4.8	Normal		88		32.3
		Bled 25 cc.	0.5	88		32.0
		Bled 25 cc.	0.5	96		32.3
		Bled 25 cc.	0.5	96		31.4
		Bled 25 cc.	0.5	120		26.1
H 35	4.7	Normal		96	94	38.2
		Bled 25 cc.	0.5	90	96	40.1
		Bled 25 cc.	0.5	93	110	39.8
		Bled 25 cc.	0.5	108	94	37.8
		Bled 25 cc.	0.5	120	112	32.5

about 15 minutes. A 10 per cent loss of blood reduced the specific gravity from 1.056 to 1.054, which indicates a dilution of about 4 per cent.

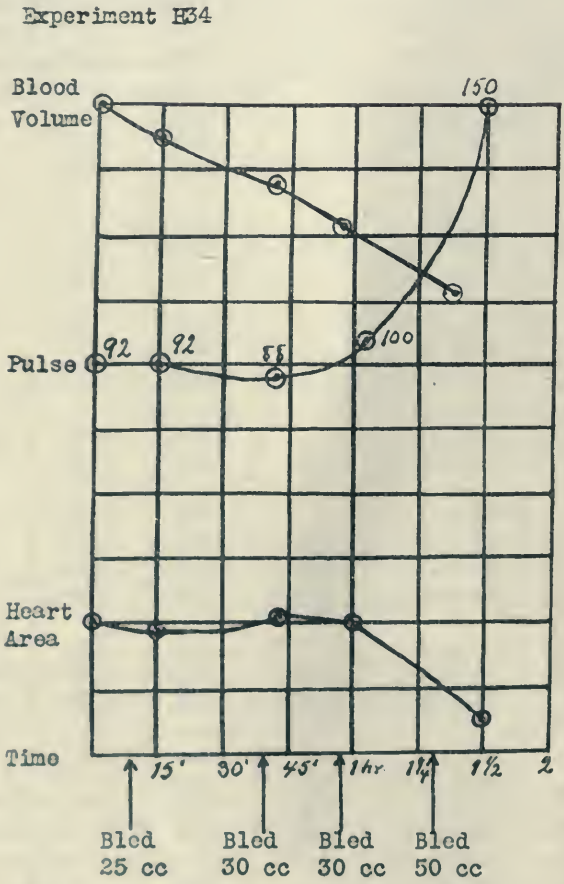

Fig. 1. Curves showing the changes in blood volume, pulse rate and heart size which occurred after four successive hemorrhages. The first determinations are the normals. Changes from normal are in per cents, 10 per cent for each line unless the actual figures are inserted. The maintenance of heart size after a loss of nearly 20 per cent of the blood volume is to be noted.

In a number of our experiments we have followed the dilution of the blood by means of hemoglobin and specific gravity determinations. Figure 2 shows that in one experiment the hemoglobin had been reduced only 6 per cent at a time when the blood volume had been decreased

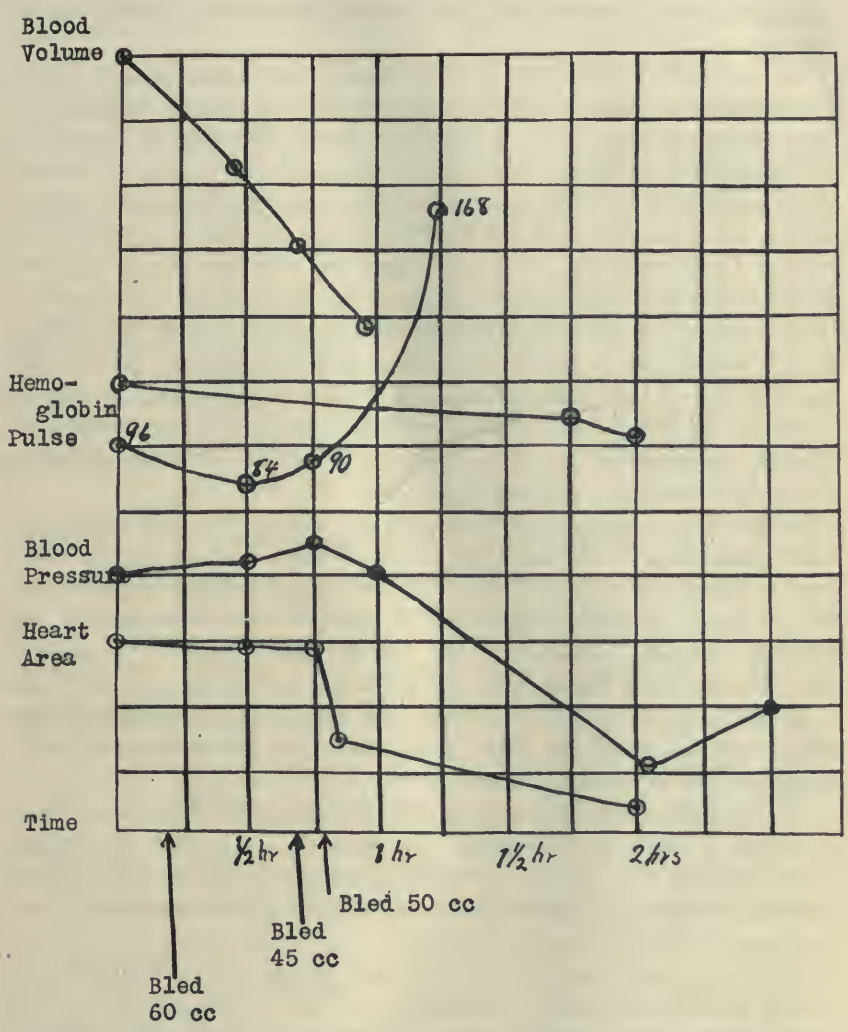

Fig. 2. Curves showing the changes in blood volume, hemoglobin, pulse rate, blood pressure and heart size after three successive hemorrhages. Determinations are plotted as in figure 1. The maintenance of heart size after hemorrhages amounting to nearly 30 per cent of the blood volume is very striking. At this time the pulse rate was within 6 beats of normal and the blood pressure had increased 5 per cent. The third bleeding destroyed the compensating mechanism and the diastolic heart size decreased at once.

40 per cent. Other experiments showed somewhat greater dilutions but in none was the inflow of tissue fluid during the period of the experiment sufficient to restore the blood volume to anything like its normal value.

In agreement with Richet we have found that specific gravity determinations give evidence of dilution earlier than can be obtained by hemoglobin readings. For determining specific gravity we have used Hammerschlag's method. In three experiments in which the hemorrhage equaled 15 to 25 per cent of the blood volume, the specific gravity during the time necessary for x-ray examinations fell only 0.002, that is from 1.054 to 1.052. This would indicate that less than 5 per cent of the blood volume had been replaced by tissue fluids. Neither the literature nor our own observations would seem to justify attributing to the absorption of tissue fluid more than a secondary part in the maintenance of the cardiac diastolic size after hemorrhage. It may be worth noting that in rabbits blood volume is more quickly restored than in dogs, a conclusion we have arrived at from the literature and confirmed by several experiments of our own.

If after hemorrhages amounting to 2 per cent of the body weight the heart maintains its diastolic size it would seem that up to this point cardiac filling was not interfered with and that the output per beat was still normal. It might, however, be that the extent of systole was reduced and that, undetected by diastolic x-ray picture, the output had actually been falling with the decreased blood volume. In order to investigate this point we studied the effects of hemorrhage in the anesthetized dog with the chest open by means of the cardio-plethysmograph and blood pressure manometers.

Cardio-plethysmographic and blood pressure records after hemorrhage. In tables 2 and 3 are presented the complete data from two experiments showing the effect of successive hemorrhages on cardiac output, venous pressure and arterial pressure. Eight other experiments have been made with quite similar results.

It will be seen from these tables that during and immediately following small hemorrhages amounting to less than 1 per cent of the body weight, the cardiac output per minute is always, and systolic, diastolic and pulse pressures are usually, slightly decreased. Within a few minutes or even less, however, recovery of the normal is generally complete.

In this series of eight experiments the cardiac output per minute never failed to be reduced immediately after a small hemorrhage.

The reduction, so long as the blood lost was under 1 per cent of the body weight, was not great. It was brought about by a decrease in output per beat rather than by changes in heart rate. The limited

TABLE 2

Data from experiment 5 showing the effect of five successive hemorrhages

TIME	PROCEDURE	PULSE RATE	VENOUS PRESSURE	CARDIAC OUTPUT		ARTERIAL BLOOD PRESSURES			
				Per minute	Per beat	Systolic	Diastolic	Mean	Pulse pressure
2.39	Normal	114	8.0	2780	24.4	119	56	70	63
2.45	After bleeding 0.2 per cent of body weight	102	7.75	2505	24.6	120	56	68	64
2.48	Three minutes after bleeding	102	7.75	2794	27.3	127	62	74	65
2.50	Second bleeding total of 0.40 per cent body weight	114	7.5	2540	22.8	124	59	73	65
2.53	Three minutes after second bleeding	114	7.0	2979	26.1	131	63	75	68
2.56	Third bleeding total of 0.67 per cent body weight	114	7.0	2547	22.3	114	54	72	60
3.07	Eleven minutes after third bleeding	120	8.0	2479	20.6	140	76	92	64
3.22	Twenty-six minutes after third bleeding	120	8.0	2492	20.8	108	49	63	58
3.30	Fourth bleeding total of 0.8 per cent body weight	156	6.75	968	6.2	101	61	72	40
3.38	Eight minutes after fourth bleeding	162	6.25	1160	7.2	107	63	71	44
3.41	Fifth bleeding total of 1.1 per cent body weight	162	6.25	1071	6.9	88	48	58	40
3.44	Three minutes after fifth bleeding	162	6.25	1084	6.9	85	50	60	35

extent of the reduction, amounting only to 2 or 3 cc. per beat, together with the rapid recovery, prevented us from detecting this change by means of the x-ray. In a very few minutes the minute output returned not only to normal but usually well above.

TABLE 3

Data from experiment 7 showing the effect of nine successive hemorrhages

TIME	PROCEDURE	PULSE RATE	VENOUS PRESSURE	CARDIAC OUTPUT		ARTERIAL BLOOD PRESSURE			
				Per minute	Per beat	Systolic	Diastolic	Mean	Pulse pressure
2.55	Normal	138	9.5	1642	11.9	95	50	58	45
3.02	Bled 0.13 per cent body weight	138	9.5	1371	9.9	93	53	60	40
3.06	Four minutes later	138	10.0	1820	13.2	115	67	72	48
3.07	Second bleeding total of 0.28 per cent body weight	138	10.25	1629	11.8	117	63	73	54
3.09	Two minutes later	132	10.25	1679	12.7	109	59	72	50
3.14	Third bleeding, total of 0.43 per cent body weight	144	9.75	1442	10.0	107	65	68	42
3.18	Four minutes later	144	10.5	1771	12.3	119	73	83	46
3.24	Ten minutes after third hemorrhage	150	9.5	1841	12.2	118	79	86	39
3.34	Fourth bleeding total of 0.60 per cent body weight	144	9.25	1504	10.4	120	66	78	54
3.39	Five minutes later	156	9.5	1722	11.1	123	70	83	53
3.41	Fifth bleeding total of 0.75 per cent body weight	150	9.5	1614	10.7	118	66	74	52
3.45	Four minutes later	144	8.75	1629	11.3	125	70	80	55
3.46	Sixth bleeding total of 0.92 per cent body weight	150	8.75	1387	9.3	112	64	71	58
3.51	Five minutes later	144	8.5	1295	8.6	123	68	82	55
3.54	Seventh bleeding of 1.2 per cent body weight	144	8.0	1283	8.9	102	65	71	37
4.02	Eight minutes later	144	8.25	1216	8.4	105	64	75	41
4.06	Eighth bleeding 1.5 per cent body weight	150	8.5	829	5.5	98	52	68	46
4.11	Five minutes later	150	8.5	989	6.6	102	56	65	46
4.13	Ninth bleeding 1.8 per cent body weight	150	7.75	622	4.4	80	44	52	36
4.21	Eight minutes later	150	7.5	730	4.8	88	46	60	42
4.41	Twenty-eight minutes after ninth hemorrhage	150	7.75	725	4.8	76	28	48	48

Arterial blood pressures were somewhat more inconstant than the volume outputs. The result of the first bleedings was often a tendency to raise systolic pressure either at once or during the next few minutes. Both of the experiments submitted illustrate this point. Later there was a slight fall of systolic pressure with a prompt recovery. Venous pressure showed no significant changes.

When the amount of bleeding averaged from 0.75 to 1 per cent of the body weight the usual slight reduction in output appeared after the hemorrhage but the recovery was not as complete as previously. For example, in experiment 5, table 2, 3 minutes after the second bleeding the minute output exceeded the normal by nearly 200 cc., but after the third bleeding the recovery was not quite to normal.

When the amount of blood drawn exceeded about 1.2 per cent of the body weight the cardio-vascular reactions differed from those above described. The decrease in output per minute and per beat became particularly marked. The systolic and pulse pressures were considerably lowered. The diastolic and mean pressures, however, usually held up near normal until a later bleeding. Venous pressure was permanently lowered. These changes may be noted as the result of the fourth bleeding in table 2 and the eighth bleeding in table 3.

It would seem that the cardio-vascular reactions to hemorrhage as determined particularly by output fell into three stages. First after bleedings not exceeding on the average 0.7 per cent of the body weight there is quick recovery with over-compensation, that is an actual increase in output. Second, after bleeding from 0.7 to 1 per cent of the body weight there is a return of the output to normal but the rebound above normal, so marked in the first stage, is lacking. One is tempted to assume that some compensating mechanism has reached its limit and that this is a critical stage. Third, after hemorrhages amounting to 1.2 per cent of the body weight or more there is failure to increase the output anywhere near to normal. These figures are of course only approximate, varying in different animals, but the stages themselves were apparent in all the experiments.

The effect of hemorrhage on the capillaries and venules. So far we have shown that, except for a very brief period immediately after bleeding, the heart may retain its diastolic size and keep up its usual minute volume output until there is a total hemorrhage of about 2 per cent of the body weight in the intact animal under morphia, and about 1.2 per cent when the heart is exposed under anesthesia and artificial respiration. Since in the dog the total amount of blood is nearly 10 per cent

of the body weight (9), (10), these figures indicate that in the intact animal during a reduction of approximately 20 per cent in blood volume the heart may still keep up its normal output. To do this the right heart must continue to receive its usual supply of blood, for the systolic output certainly depends directly on the inflow during diastole. The problem resolves itself into finding an explanation for the adequate blood supply to the right heart. That the body fluids cannot serve as a source in the time required we have already shown. Theoretically the conditions might be met if the velocity of the venous return were sufficiently augmented. For this, however, there is no evidence, since the venous pressure remained unchanged. The same fact would seem to preclude a decrease in the capacity of the pulmonary bed sufficient to compensate for the hemorrhage. The portal circulation with its double resistance is particularly fitted to serve as a reservoir for the venae cavae, as pointed out by Krogh (11), but constriction of arterioles is necessary to render this supply available. The failure of systolic blood pressure uniformly to increase after the bleedings seems to exclude this explanation. The recent work of Hooker (12) on the functional activity of the venules and capillaries has called our attention to a possible solution of the problem. If there are venules or capillaries containing blood, but not in the active course of the circulation, a constriction of these vessels would add fluid and tend to maintain the effective circulation. Cardiac output, after a brief period for the readjustment, might be maintained so long as the content of stagnant venules and capillaries sufficed to meet the loss from hemorrhage. That there are such capillaries and venules we know from the work of Roy and Brown (13) and Danzer and Hooker (14). The latter discuss fully the literature of this very interesting subject.

To find if there is any evidence for such a hypothesis, we have observed the venules and capillaries in the ears of dogs before, during and after hemorrhage. For this purpose we have used the method of illumination described by Hooker (12). Three animals have been studied. Dog 1 weighed 3.65 kilos. After bleeding 100 cc. or 2.7 per cent of his body weight the blood vessels slowly decreased during the next 3 minutes. That this was due to the hemorrhage could scarcely be doubted for the flow had continued for sometime previously. Dog 2 weighed 8 kilos. He was bled 100 cc. with no observable effect. On a second bleeding, when the hemorrhage had reached a total of 160 cc., there was an almost instantaneous whitening of the ear caused by the constriction of the venules and capillaries, both of those that had been

very active, and those that had been less so or entirely stagnant. There was thus a noticeable effect on the blood vessels when the bleeding equaled 2 per cent of the body weight. Dog 3 weighed 10.8 kilos and was bled 216 cc. Photomicrographs were taken immediately before and after the hemorrhage. These are reproduced in figure 3. The constriction of the venules and capillaries is very striking.

These experiments are clear proof that at a certain stage of hemorrhage there is some kind of a circulatory reaction resulting in the

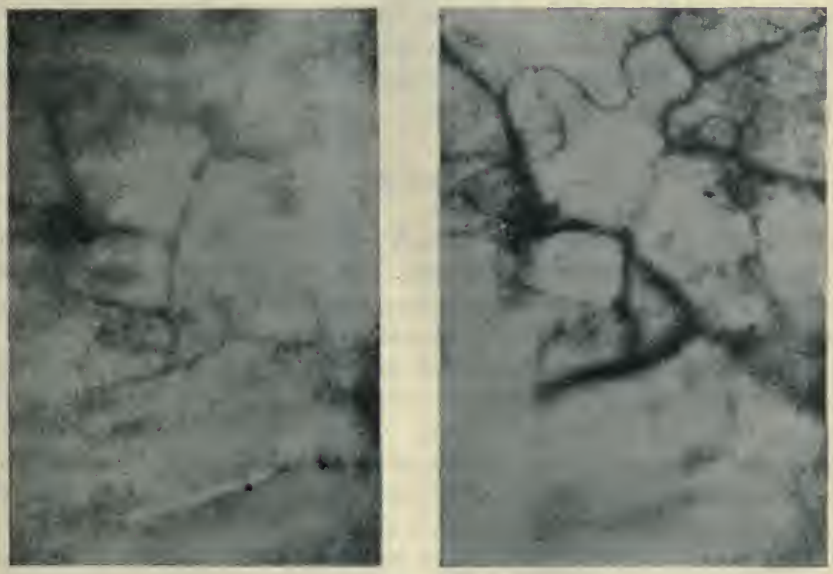

Fig. 3. Photomicrographs of the surface of a dog's ear before and after a hemorrhage of 215 cc. or 2 per cent of the body weight. Magnification 90 ×. Plates not retouched.

constriction of the venules and capillaries in the skin. All the indications are that the response is active and not passive. Whether or not it is brought about through nervous connections has not been investigated. The most obvious purpose served by this reaction would be to furnish an adequate supply of blood for the venous return to the right heart.

It is realized that the constriction of vessels in the skin could not make up for a very large hemorrhage since only some 2 or 3 per cent of the total blood volume is normally found at the surface of the body.

What we have seen is probably the last stage in the body's attempt to keep up the effective circulation. At first the stagnant vessels in the muscles or organs of the splanchnic area are probably called upon. Later when the circulation is at the breaking point, as it is when the bleeding equals 2 per cent of the body weight, venules and capillaries are constricted in widespread areas. In a word, the fact that at a certain stage of hemorrhage the venules and capillaries are seen to constrict justifies the hypothesis that there may have been initial constriction, particularly of stagnant vessels, which would thereby add a sufficient volume of blood to preserve the status quo of the effective circulation. It is believed that by some such means as this the heart is enabled to keep up its minute volume output until the hemorrhage reaches relatively large proportions.

SUMMARY

The effect of hemorrhage on the diastolic heart size of the dog has been studied by means of the x-ray. In anesthetized animals under artificial respiration the cardiac output and venous pressures have been determined after bleeding by cardiometers and manometers.

In the intact animal hemorrhage amounting to about 2.1 per cent of the body weight is necessary before the diastolic heart size, and presumably the output, is reduced. In the anesthetized animal with open chest, the minute volume output is maintained, with the exception of a slight drop immediately after bleeding, until the total hemorrhage equals about 1.2 per cent of the body weight.

Various mechanisms which might account for maintenance of cardiac output under these conditions are discussed. The only satisfactory explanation seems to be that the effective circulation is kept up by constriction of venules and capillaries, particularly those which have been more or less stagnant. Evidence in support of this idea is submitted. When hemorrhage in the intact animal reaches about 2 per cent of the body weight, the venules and capillaries of the ear may be seen markedly to constrict. Since such a mechanism is thus shown to exist, it becomes probable that it may have been operating in various parts of the body in earlier stages of the hemorrhage and that in this way there was provided a constant venous return and cardiac output even though the blood volume was decreased.

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FURTHER EXPERIMENTS ON THE ACTIVATION OF MUSCLE CATALASE BY LIVER[1]

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Received for publication December 31, 1920

In 1918 I published an account of some observations on catalase in which it was shown that when liver and muscle act together on hydrogen peroxide, the amount of gas given off is in excess of the sum of the two acting separately (1). Loevenhart (2), in a similar observation in 1905, attributed it to a neutralizing effect on the retarding action of the acid, and argued against the presence of a kinase. About the same time Battelli and Stern (3) described a philocatalase which had the property of antagonizing anticatalase and of regenerating catalase. They also described an "activator" of philocatalase. Some earlier experiments of my own seemed to show that muscle acted as well in acid as in neutral peroxide, and this, taken with other reasons, led me to the tentative suggestion that there might be an accelerator secreted by the liver. At this point the work was brought to an abrupt termination by absence from the University and the question of an accelerator was left for the future. Upon my return I again took up the problem, but was unable to get results that were consistent. Only occasionally could acceleration be obtained in acid H_2O_2. Meanwhile Takeda (4) pointed out that the acceleration in neutral peroxide was absent in many cases. He also found that acceleration occurred in acid H_2O_2 when liver was combined with boiled muscle, egg white or peptone solution. Takeda suggests that the accelerating effect is due to some organic substance contained in the tissue; possibly a "protective colloid."

Having convinced myself that the hypothesis of a liver hormone is untenable, the following results are offered as a somewhat more definite explanation of the phenomenon than those hitherto suggested.

[1] The expenses of this research were paid out of a grant from the Research Board of the University of California.

Method. Granted an acceleration of catalytic activity when liver and muscle are placed in acid H_2O_2, and no acceleration when placed in neutral H_2O_2, it does not follow that there is no accelerator secreted by the liver. It may be one which acts in an acid medium and not in a neutral. Obviously the thing to do is to separate the two if possible, and with that in view catalase was prepared from rabbits' liver and muscle by a method which is a combination of that of Battelli and Stern (5) for catalase, and Van Slyke and Cullen (6) for urease. (My attention was called to the latter by my colleague, C. L. A. Schmidt of the Department of Biochemistry.) The rabbits were perfused through the aorta with m/6 NaCl until the fluid ran white from the veins. The tissue was reduced to a pulp in a meat grinder and shaken for an hour or so with twice its volume of distilled water. It was then strained through linen in a press and the pulp subjected to a second extraction with its own volume of distilled water, the two watery extracts were added together and poured slowly into "a volume of acetone so large that the enzyme undergoes practically instant dehydration." (Van Slyke and Cullen state that the volume of acetone should not be less than ten times that of the water extract, but I have obtained excellent preparations of catalase with three or four volumes.) The dense precipitate was filtered through hardened filter paper. After evaporating the acetone from the precipitate by a current of air, the precipitate was mixed with two volumes of distilled water and either shaken for an hour or two or allowed to stand for several hours. It was then filtered, and the filtrate precipitated with acetone as before. After filtering through hardened filter paper, the precipitate was dried in a vacuum over sulphuric acid, and pulverized in a mortar. This gives a catalase impure of course, which is very active. Twenty milligrams of liver catalase thus prepared from rabbit's liver gave off 69 cc. of oxygen from neutral hydrogen peroxide in 10 minutes. Twenty milligrams of muscle catalase prepared from the same rabbit gave 12 cc. of oxygen in 10 minutes. Two hundred grams of liver yielded about 3 grams of catalase, while 200 grams of muscle gave but 0.9 gram. The amounts vary, however, with the individual.

Other methods have been tried, such as precipitation with ether and with alcohol, but the above method has yielded a more soluble preparation in my hands. Incidentally it may be said that precipitation with ether does not destroy the catalase, although Burge (7) states that liquid ether will destroy catalase very quickly.

Results. When liver catalase and muscle catalase act together on acid or neutral H_2O_2 there is practically no acceleration. The slight acceleration shown in table 1 is within the experimental error of the method used, as Becht (8) has pointed out. The catalase was prepared as described in the text. The volume of oxygen is reduced to 0° and

TABLE 1

	SUBSTANCE	QUANTITY	H_2O_2 REACTION	O_2	VOLUME INCREASE	ACCELERATION
		grams		*cc.*		*per cent*
1	Muscle catalase	0.02		6		
	Liver	0.02	Acid	23	+1	+3.0
	Muscle catalase 0.02 gram and liver catalase	0.02		30		
2	Muscle catalase	0.02		6		
	Liver	0.02	Acid	27	0	0
	Muscle catalase 0.02 gram and liver catalase	0.02		33		
3	Muscle catalase	0.02		4		
	Liver	0.02	Acid	14	−1	−5.5
	Muscle catalase 0.02 gram and liver catalase	0.02		17		
4	Muscle catalase	0.02		11		
	Liver	0.02	Neutral	69	+3	+4.0
	Muscle catalase 0.02 gram and liver catalase	0.02		83		
5	Muscle catalase	0.02		4		
	Liver	0.02	Neutral	47	+2	+4.0
	Muscle catalase 0.02 gram and liver catalase	0.02		53		
6	Muscle catalase	0.02		6		
	Liver	0.02	Neutral	56	+2	+3.0
	Muscle catalase 0.02 gram and liver catalase	0.02		64		

760 mm. Hg. taking the nearest whole number. The H_2O_2 was neutralized with N/10 NaOH until it had a pH 7 by the Clark and Lubs scale. In all cases the time is 10 minutes' shaking. The temperature was constant for any given series.

At first sight these results seem to indicate that something in the nature of an accelerator had been removed from the liver catalase; but

TABLE 2

	SUBSTANCE	QUANTITY	H_2O_2 INITIAL pH	O_2	VOLUME INCREASE	ACCELERATION	H_2O_2 FINAL pH
				cc.		*per cent*	
1	Muscle	0.5 gm.	2.4	6	+17	+113	4.5
	Liver	0.1 gm.	2.4	9			2.4
	Muscle 0.5 gram and liver	0.1 gm.	2.4	32			4.5
2	Muscle	0.5 gm.	2.4	6			4.5
	Egg white (boiled)	0.5 gm.	2.4	0	+1	+17	4.5
	Muscle 0.5 gram and egg white	0.5 gm.	2.4	7			4.8
3	Muscle	0.5 gm.	2.4	6			4.5
	Serum (rabbit)	0.5 cc.	2.4	0	+2	+33	4.6
	Muscle 0.5 gram and serum	0.5 cc.	2.4	8			4.8
4	Muscle	0.5 gm.	2.4	6			4.5
	Muscle	0.5 gm.	2.4	7	+6	+46	4.5
	Muscle	1.0 gm.	2.4	19			4.8
5	Muscle	0.5 gm.	2.4	9			4.5
	Liver catalase	0.02 gm.	2.4	6	+7	+47	2.4
	Muscle 0.5 gram and liver catalase	0.02 gm.	2.4	22			4.6
6	Muscle (boiled)	0.5 gm.	2.4	0			3.6
	Liver catalase	0.02 gm.	2.4	5	+4	+80	2.4
	Muscle 0.5 gram and liver catalase	0.02 gm.	2.4	9			3.6
7	Liver	0.1 gm.	2.4	16			2.4
	Egg white (boiled)	0.5 gm.	2.4	0	+4	+25	4.5
	Liver 0.1 gram and egg white	0.5 gm.	2.4	20			4.5
8	Liver	0.1 gm.	2.4	15			2.4
	Serum (rabbit)	0.5 cc.	2.4	0	+8	+53	4.6
	Liver 0.1 gram and serum	0.5 cc.	2.4	23			4.8
9	Liver	0.1 gm.	2.4	14			2.4
	Liver	0.1 gm.	2.4	14	+4	+14	2.4
	Liver	0.2 gm.	2.4	32			2.6
10	Muscle catalase	0.02 gm.	2.4	8			2.4
	Liver (fresh)	0.1 gm.	2.4	58	0	0	2.4
	Muscle catalase 0.02 gram and liver	0.1 gm.	2.4	66			2.4

TABLE 2—*Concluded*

	SUBSTANCE	QUANTITY	H_2O_2 INITIAL pH	O_2	VOLUME INCREASE	ACCELERATION	H_2O_2 FINAL pH
				cc.		*per cent*	
11	Casein (neutral)	0.5 gm.	2.4	0			3.6
	Liver catalase	0.02 gm.	2.4	7	0	0	2.4
	Casein 0.5 gram and liver catalase	0.02 gm.	2.4	14			3.6
12	*Na caseinate	0.5 gm.	2.4	0			5.6
	Liver catalase	0.02 gm.	2.4	5	+60	+1200	2.4
	Na caseinate 0.5 gram and liver catalase	0.02 gm.	2.4	65			5.6

* The Na caseinate was made by dissolving 1 gm. casein in 8 cc. of $\frac{N}{10}$ NaOH. It remains colorless on the addition of phenolphthalein (9).

referring to table 2 it will be seen that liver catalase plus muscle tissue gives acceleration. Loevenhart found acceleration with boiled liver, and Takeda used boiled muscle and even egg white with similar results. I have found acceleration not only with boiled white of egg, but also with blood fibrin and with gelatin. Now these substances are all proteins, and therefore attention was turned to their effect on the H-ion concentration of the peroxide. The hydrogen ion concentration of the peroxide was determined by Clark and Lubs scale very kindly prepared for me by Mr. S. B. Randall of the Department of Pathology. The substance was then added and after 10 minutes' shaking the pH of the solution again determined. Table 2 gives the results.

Note that in all cases of acceleration there is a shifting of the pH toward the neutral point. Where the muscle acts alone the pH of the solution is changed from 2.4 to 4.5, while the liver, on account of its small bulk does not perceptibly change the H ion concentration. When the two are added together, however, the liver catalase acts on H_2O_2 with a pH of 4.5 instead of 2.4 and therefore more oxygen is given off. That this is so is shown by the following:

0.02 gram liver catalase, H_2O_2 pH 2.4 gave 3 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 3.8 gave 8 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 5.0 gave 38 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 7.3 gave 48 cc. O_2

Liver catalase was used because it does not of itself perceptibly change the pH of the H_2O_2 in the small quantities used.

CONCLUSION

It would seem then, that we must abandon the hypothesis of a liver hormone and conclude that at least one of the factors of the acceleration is a change in pH of the H_2O_2, brought about by the action of the proteins and possibly salts also. If we take Loevenhart's "neutralization" and Takeda's "protective action" in a broad sense, they are in agreement with the above. Whether this is a "buffer" action of the proteins or an adsorption phenomenon is not within the scope of this paper to determine.

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THE SOURCE OF DIASTASES OF THE BLOOD

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Received for publication January 10, 1921

In doing some work on chloroform anesthesia we found certain changes in the blood diastases. Knowing that chloroform injures liver cells, the question arose as to whether the blood diastases might not come chiefly from the liver, instead of the pancreas, as Gould and Carlson's work indicates (1). It was thought possible that measurements of changes in the diastase content of the blood after pancreas operation and administration of ether and chloroform might aid in determining the source of the starch-splitting enzymes of the blood serum.

Experimental work. The determination of the serum diastases was made according to the method devised by McGuigan and Von Hess (2) except the starch solution used was ½ per cent instead of 1 per cent. The essential of the method is the incubation of a measured volume of ½ per cent soluble starch solution with a measured amount of serum until no color is produced by the mixing of 1 cc. of dilute iodine-potassium iodide solution and one drop of incubated mixture. The results are expressed in minutes required for the disappearance of the color reaction.

Serums were prepared from a number of normal dogs and the diastase contents determined. The results are given in table 1.

A group of animals was operated on to remove all of the pancreas except the tip of the tail which was implanted with its own blood supply under the skin of the abdomen. Determinations were made on these animals 3 days in succession after they had well recovered from the operation. The average results for each animal are given in table 1.

After observations were completed on the animals from which the pancreas had been partially removed, the remaining fragment of pancreas was taken away. After several days, a series of determinations on the diastase content of the serums of these dogs was made. The

results are given in table 1, the average of several days' observations being taken.

The immediate effects of anesthesia on the blood diastases were determined on several groups of dogs. A group of animals with healed partial pancreatectomies was given 15 minutes of ether anesthesia. The blood diastases were measured before and after this procedure. A second group of dogs with healed complete pancreatectomies was subjected to the same tests. A third group of dogs was given chloroform and the diastases measured before and after 15 minutes of anesthesia. These were normal animals. A group of partially pancreatectomized

TABLE 1

The effect of partial and complete removal of the pancreas on blood diastases (expressed in minutes)

NORMAL DOGS	DOGS PARTIALLY PANCREATECTOMIZED	DOGS COMPLETELY PANCREATECTOMIZED
24	26.5	55.5
26*	9.0	30.5
18	24.2	34.0
21	14.5	27.0
20		26.6
27		27.0
21		
13		
36		
23		
22		
11		
13		
Average 21.1	18.5	33.4

* Dog had pancreatic cyst.

dogs was given chloroform and the tests made. The same was done with a series of completely pancreatectomized animals. The results of the above procedures are given in table 2.

The continued effect of anesthesia on the blood diastases of several groups of dogs was determined. After taking the blood and measuring the diastase concentration of a group of animals, they were anesthetized with ether for half an hour. The following day they were bled again and the diastase determined. Another group of normal dogs was subjected to the same treatment except chloroform was used in place of ether. A third group of normal dogs was given a fast of 2 days and

TABLE 2

Immediate effect of anesthesia and pancreatectomy on blood diastases

ANIMAL	PANCREAS OPERATION	ANESTHESIA	DIASTASE TIME (MINUTES)	
			Before anesthesia	After anesthesia
1	Partial removal	Ether	24	24
2	Partial removal	Ether	6	6
3	Partial removal	Ether	16	14
4	Partial removal	Ether	14	14
5	Partial removal	Ether	24	23
1	Complete removal	Ether	54	51
2	Complete removal	Ether	31	30
3	Complete removal	Ether	17	17
9	No removal	Chloroform	21	33
10	No removal	Chloroform	13	36
11	No removal	Chloroform	19	57
1	Partial removal	Chloroform	24	24
2	Partial removal	Chloroform	6	6
3	Partial removal	Chloroform	17	16
4	Partial removal	Chloroform	15	15
1	Complete removal	Chloroform	57	58
2	Complete removal	Chloroform	30	28
3	Complete removal	Chloroform	17	16

TABLE 3

Prolonged effect of anesthesia on blood diastases

ANIMAL	CONDITION	ANESTHETIC	DIASTASE TIME (MINUTES)	
			Before anesthesia	After anesthesia
12	Normal	Ether	5	6
13	Normal	Ether	23	19
14	Normal	Ether	22	15
15	Normal	Ether	11	11
16	Normal	Ether	13	13
17	Normal	Chloroform	14	21
18	Normal	Chloroform	20	26
19	Normal	Chloroform	15	24
20	Normal	Chloroform	19	25
21	Normal	Chloroform	16	23
22	Fasted	Chloroform	9	12
23	Fasted	Chloroform	20	31
24	Fasted	Chloroform	13	23
25	Fasted	Chloroform	16	34
26	Fasted	Chloroform	16	23
27	Fasted	Chloroform	15	30

tested in the same way as the second group. The results of these tests are given in table 3.

The group averages are given in table 4. The values for the immediate effect of ether on the diastases of normal dogs are taken from a previous article (3). The values here stated are half those in the article because in that work the starch solution was 1 per cent and in this ½ per cent.

TABLE 4

The average immediate and prolonged effects of anesthesia and pancreatectomy on blood diastases

PANCREAS	ANESTHETIC	OBSERVATION	DIASTASE TIME (MINUTES)		
			Before anesthesia	After anesthesia	Change
Normal	Ether	Immediate	20.5	20.0	−0.5
Partial removal	Ether	Immediate	16.8	16.2	−0.6
Complete removal	Ether	Immediate	34.0	32.6	−1.4
Normal	Chloroform	Immediate	17.6	42.0	+24.4
Partial removal	Chloroform	Immediate	15.5	15.2	−0.3
Complete removal	Chloroform	Immediate	34.8	34.0	−0.8
Normal	Ether	Prolonged	14.8	12.8	−2.0
Normal	Chloroform	Prolonged	16.8	23.8	+7.0
Fasted	Chloroform	Prolonged	14.8	25.5	+10.7

DISCUSSION

Schlessinger (4) removed the pancreas from dogs and found a decrease of blood disatases. He concluded that this enzyme originated in the pancreas.

Carlson and Luckhardt (5) removed the pancreas and obtained no change in blood diastases. They found a slight decrease in them after ether anesthesia.

Otten and Galloway (6) from extirpation experiments concluded that the pancreas is responsible directly or indirectly for a large portion of the diastases in the blood. They suggest the possibility that diastases in the liver cells may take some part in the splitting of glycogen that later passes into the blood stream.

Gould and Carlson (1) by operations on the pancreas have obtained results which lead them to conclude that ligation of the outlets of the pancreas increases the blood diastases and removal of the pancreas decreases the blood diastases.

Ross and McGuigan (3) made observations on the diastase changes brought on by ether anesthesia with variation in diet. Ether anesthesia caused no change in the blood diastases of dogs fed on either a meat or a mixed diet.

The work we desired to do required that the pancreas be removed completely from a number of dogs. The removal was done in two operations. The two-stage operation was for the purpose of producing the least amount of disturbance in the animal. Since the material was available, confirmatory tests were made to see the effect of the removal of the pancreas on the blood diastases. The results given in table 1 show the average diastase time as 21.1 minutes for normal animals, 18.5 minutes for partially pancreatectomized dogs and 33.4 minutes for dogs without any pancreas tissue. This confirms the findings of Schlessinger and Gould and Carlson, that the removal of the pancreas decreases the blood diastases.

The effect of ether anesthesia on the blood diastases of animals with varying amounts of pancreas was measured. The table of averages is number 4. Anesthesia of normal dogs with ether changed the diastase time from 20.5 to 20.0 minutes, of partially pancreatectomized dogs changed the diastase time from 16.8 minutes to 16.2 minutes, and dogs without any pancreas from 34.0 minutes to 32.6 minutes. The changes are considered negligible.

The effect of chloroform anesthesia on dogs with varying amounts of pancreas was distinctive. The average results are given in table 4. Normal dogs had their diastase time changed from 17.6 minutes to 42.0 minutes. This marked decrease in diastases in normal dogs due to chloroform anesthesia might be caused by either one or both of two things, i.e., liver injury, if the liver is a source of diasatases, and pancreas injury. It is commonly known that chloroform injures liver cells. Whipple and Sperry (7) found chloroform capable of producing considerable injury to the cells of the pancreas. Partially and completely pancreatectomized dogs did not have an appreciable change produced by chloroform anesthesia. This finding rules out the possibility that the liver injury was the cause of the change in blood diastases resulting from chloroform anesthesia and leaves us to conclude that the injury to the pancreas was the factor that reduced the diastases.

To test the influence of ether and chloroform further two groups of animals were given a half-hour anesthesia one day and the diastases measured before the anesthesia and the following day. One group of animals was anesthetized with ether, the other with chloroform. The

results are given in table 3. Since a fast preceding a chloroform anesthesia increases the injury to liver cells from the drug action (8), it is reasonable to suppose that the injury elsewhere, such as in the pancreas, would be likewise influenced. Therefore another group of dogs was subjected to a fast of 2 days and then given chloroform as before and diastase determinations made before the anesthesia and the following day. The results are given in table 3. The averages from table 3 are given in table 4. Ether anesthesia produced an average change from 14.8 minutes to 12.8 minutes in 24 hours. The change is negligible. A half-hour chloroform anesthesia changed the diastase time from 16.8 minutes to 23.8 minutes the following day, increasing the time 7 minutes. The group of animals fasting 2 days had their diastase time changed from 14.8 minutes to 25.5 minutes the following day, increasing the time 10.7 minutes.

Chloroform in these two groups of dogs again produced marked decreases in the blood diastases. In the group of animals subjected to a fast in which we would expect greater injury to the pancreas produced by chloroform, this drug brought about a greater decrease in the blood diastases than was the case with normal animals. These results again suggest that the pancreas is the chief source of the starch-splitting enzymes of the blood serum.

SUMMARY AND CONCLUSIONS

A group of 13 normal dogs was tested as to the diastatic power of their serums.

Groups of dogs which still retained part of the pancreas were tested 3 successive days as to the diastatic power of the blood. They were given ether anesthesia for 15 minutes and the diastases of the blood determined before and after. They were given chloroform anesthesia for 15 minutes and the same measurements were made.

Groups of dogs from which the pancreas had been completely removed were subjected to tests. The diastase content of the blood was determined. They were given 15 minutes of ether anesthesia and the diastases measured before and after the anesthesia. They were given 15 minutes of chloroform anesthesia and the same measurements made.

Three groups of animals were used to determine the effect of ether, chloroform and chloroform preceded by fasting upon the diastase content of the blood the following day. Anesthesia in each case was 30 minutes in length.

The results of the tests were as follows:

1. The removal of the pancreas decreased the blood diastases markedly.

2. Ether anesthesia had no effect on the diastases of either normal, partially pancreatectomized dogs or dogs with no pancreas.

3. Chloroform produced a marked fall in the blood diastases of normal dogs. It did not produce any appreciable change in animals whose pancreas had been partially or completely removed.

4. Half an hour of ether anesthesia produced no change in the diastases found present the following day.

5. Fasting before chloroform anesthesia increased the effect of the drug.

The preceding results with the findings of others quoted above lead us to conclude that the pancreas is practically the only source of the blood diastases.

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CHANGES IN THE CATALASE CONTENT DURING THE LIFE CYCLE

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Received for publication January 11, 1921

It is known that oxidation is low in the unfertilized egg and that it increases after fertilization (1). It is also known that oxidation or metabolism is low in the newly born, that during youth it increases rapidly to a maximum, and gradually declines throughout adult life to become almost as low in extreme old age as at birth (2). There is much evidence that whenever oxidation is increased or decreased in animals as well as in plants there occurs a corresponding increase or decrease in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide (3).

The object of the present investigation was to determine if there is an increase or decrease in catalase corresponding with the increase or decrease in oxidation during the different phases of the life cycle enumerated above. The animals used were mice and Colorado potato beetles. After skinning the mice the entire animals were macerated vigorously in a mortar with a small amount of sand for 5 minutes. Whole beetles were macerated in a similar manner.

Catalase determinations were made by adding the macerated material to neutral hydrogen peroxide in a bottle and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content. The amount of macerated mouse was 1.0 gram and of the beetles 0.5 gram. Maceration for more than 5 minutes produced little or no effect on the amount of oxygen liberated by the material.

The results of the determinations are shown in figures 1 and 2. It may be seen in figure 1 that 0.5 gram of unfertilized beetle eggs liberated 18 cc. of oxygen from hydrogen peroxide in 10 minutes and a similar amount of fertilized eggs liberated 35 cc. The unfertilized eggs were removed from the body cavity of the beetles and washed in a 0.9 per cent solution of sodium chloride. The fertilized eggs were collected, soon after they were laid, from the leaves of the potato plant

and washed as were the unfertilized ones. The increase in catalase in the fertilized eggs is attributed to the stimulation of the egg by the spermatozoön to an increased output of this enzyme.

It may be seen further in figure 1 that 0.5 gram of newly hatched macerated larvae liberated 280 cc. of oxygen; quarter grown larvae, 800 cc.; half grown, 1250 cc.; three-quarter grown, 1725 cc.; pupae, 1800 cc.; adult beetles, 1750 cc.; and very old beetles, 900 cc. By com-

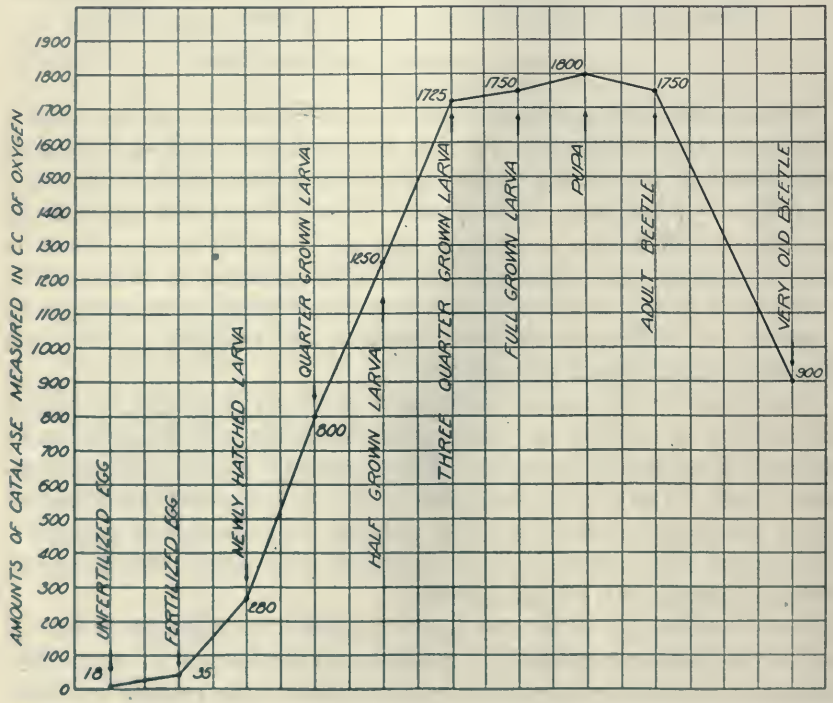

Fig. 1. The figures in the chart indicate amounts of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 gram of the macerated material.

paring these figures it may be seen that there were increasing amounts of oxygen liberated with the increase in age of the larvae and a marked decrease in catalase in the very old beetles.

In figure 2 are shown the amounts of oxygen liberated by macerated mice of different ages. It may be seen that 1.0 gram of newly born mice liberated 164 cc. of oxygen from hydrogen peroxide in 10 minutes; 24-hour mice, 190 cc.; 48-hour mice, 256 cc.; 144-hour mice, 288 cc.;

one-fourth grown mice, 435 cc.; one-half grown mice, 582 cc.; mother or adult mice, 715 cc. and very old mice, 400 cc. The very old mice were about 2 years old. By comparing these figures it may be seen that the amounts of catalase increased with an increase in age up to maturity, and that there was a decrease in catalase in the very old mice.

Determinations of the catalase content of young mice that had been deprived of food at birth were also made and it was found that the

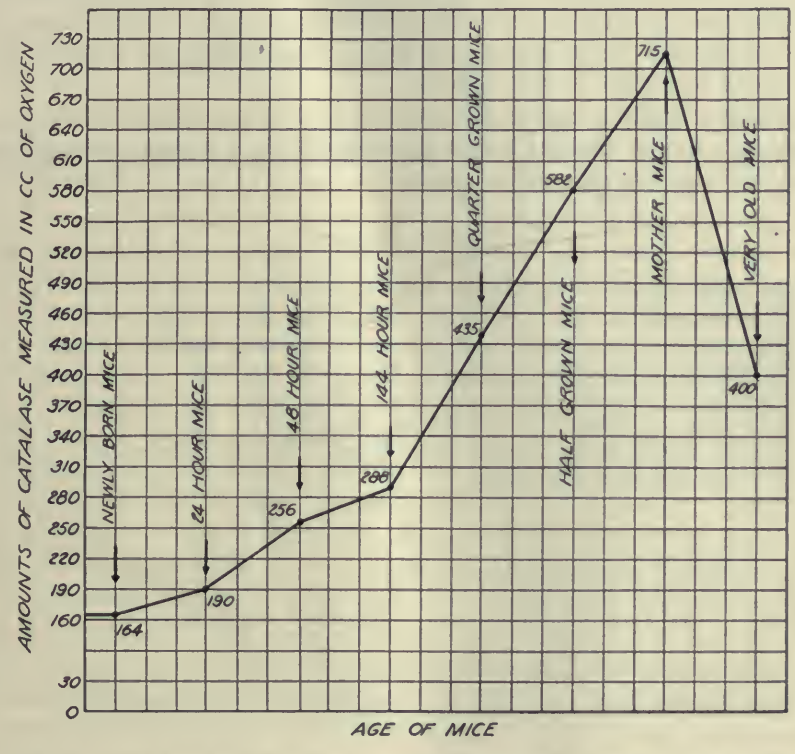

Fig. 2. The figures in the chart indicate amounts of oxygen liberated from hydrogen peroxide in ten minutes by 1.0 gram of the macerated material.

catalase content of these starved mice was not increased with an increase in age as was the case with the mice fed normally. We had already found that the ingestion of food greatly increased the blood catalase by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme. Hence the increase in catalase with increase in age is probably due to the stimulating effect of the food on the alimentary glands.

SUMMARY

The increased oxidation or metabolism with resulting development after fertilization is attributed to an increase in catalase brought about by the stimulation of the egg by the spermatozoön to an increased production of this enzyme.

The low metabolism or oxidation in the newly born is attributed to the low catalase content, while the intense metabolism characteristic of youth and adult life is attributed to the high catalase content due to the stimulation of the alimentary glands, particularly the liver, to an increased formation of this enzyme.

Similarly the low metabolism of the aged is attributed to the low catalase content brought about presumably by a lessened output of catalase from the alimentary glands.

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THE NUTRITIVE VALUE OF YEAST IN BREAD[1]

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Received for publication January 14, 1921

Yeast has been used in bread making since the earliest times and continues as the great leavening agency, although sometimes substituted in this capacity by one of a variety of baking powders depending on the liberation of carbon dioxide from inorganic carbonates. There has also been some tendency of recent years to decrease the amount of yeast used in bread making as a matter of economy.

In view of the fact that bread is the chief of staple foods, it is of importance to know whether the yeast used in its preparation should be looked upon merely as a generator of carbon dioxide or whether yeast improves the nutritive value of the loaf. If, as seems clear, yeast must of itself be considered as a food, it is of importance to know in what way it supplements the other ingredients of bread and in what amounts it should be used to obtain a product of maximum nutritive value. Also we cannot afford to underestimate the possibility of yeasts ultimately replacing in the diet certain other important foodstuffs.

It has been known for a number of years that the ash of yeast is especially rich in the important element phosphorus, containing at the same time very considerable amounts of potassium and lesser amounts of other inorganic elements essential in nutrition. Yeast also contains a considerable amount of carbohydrate, including cellulose and gum-like substances and glycogen. It is high in protein and in a nucleic acid yielding purine and pyrimidine bases, a pentose, and phosphoric acid on hydrolysis.

That yeast contains an antineuritic substance was shown by Schaumann (1). Hopkins first showed yeast to contain a substance small amounts of which accelerated the growth of rats fed purified foods (2). These observations have been repeatedly confirmed and extended

[1] The expenses of this investigation were defrayed in part by a grant from Mrs. M. H. Henderson.

until it is now generally recognized that yeast is one of the richest and cheapest sources of water-soluble vitamine.

A number of experiments have been carried out to determine the extent of digestion and absorption by men and lower animals of the protein of yeast, values of from 82 to 98.4 per cent having been obtained where yeast was merely an added food. Yeast as a sole source of nitrogen was utilized by dogs to the extent of 66 to 76 per cent according to experiments of Schill, who found in spite of this relatively low coefficient of digestibility that the protein of yeast led to a greater nitrogen retention than was obtained with the usual diet of dog biscuits (3). That yeast could be used in human nutrition to replace from 10 to 30 per cent of the nitrogen of a mixed diet was shown in this laboratory by Hawk, Smith and Holder (4), who found biscuits prepared from yeast-containing flour were attractive in flavor, (in fact preferred by the subjects to ordinary white biscuits), and that the subjects gained more weight and showed a distinctly higher nitrogen retention on the yeast-containing diet. Osborne and Mendel found that rats could grow for over a year on a diet in which yeast was the sole source of protein as well as of water-soluble vitamine (5). Karr (6) found the feeding of yeast to dogs on a diet deficient in water-soluble vitamine and possibly protein to lead to increased nitrogen retention. He also found that addition of yeast to such a diet led to an increased consumption of food by such animals. Mattill and Conklin (7) found adolescent rats fed milk only did not thrive or reproduce, but the addition of yeast led to normal growth and partially successful reproduction.

Experimental procedure. The experimental procedure was simple. Young male albino rats, obtained from the rat colony of the Wistar Institute of Anatomy, were used as experimental subjects, and were divided into two groups of eleven rats each. The attempt was made to have the two groups as nearly alike as possible at the start of the experiment. The average weights for the two groups at the start were 60 and 61 grams respectively, as is shown in chart 3.

The rats in one group were fed white bread made to approximate closely standard home-made bread, while the rats in the other group were fed bread made with considerable extra yeast. The high yeast content was obtained by mixing 5 per cent of dried compressed yeast[2] with the flour and by using six times as much fresh yeast as was used

[2] Fleischmann's compressed yeast was used. This yeast was dried below 105°C., then powdered and mixed with the wheat flour.

in making the standard bread. The recipes used in making the two kinds of bread were as follows:

Extra yeast bread	*Standard bread*
2 quarts liquid ($\frac{1}{2}$ milk, $\frac{1}{2}$ water)	$2\frac{1}{2}$ quarts liquid ($\frac{1}{2}$ milk, $\frac{1}{2}$ water)
$7\frac{1}{2}$ lbs. flour	$7\frac{1}{2}$ lbs. flour
6 oz. dried yeast	6 oz. Crisco
6 oz. Crisco	3 teaspoons salt
3 teaspoons salt	3 cakes yeast
18 yeast cakes	3 teaspoons sugar
6 teaspoons sugar	

It is necessary to decrease liquid when using additional yeast. The dried yeast flour needs additional cake yeast to "raise" it.

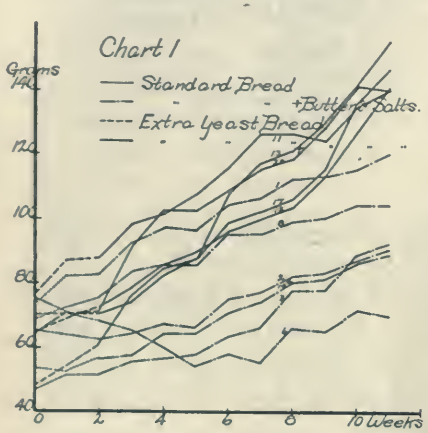
At the end of two weeks, butter and a mixture of inorganic salts (8) were added to the diet of each group. The rats were weighed each week. The weights of the individual rats are shown in charts 1 and 2. In chart 3 the results are summarized by means of average curves for the rats on standard and on extra yeast bread.

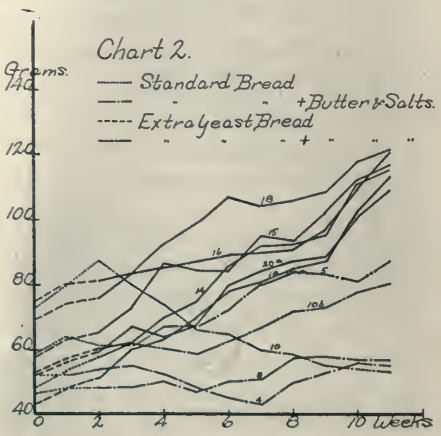
It will be noted that on the average the rats fed extra yeast bread gained 66 grams in 11 weeks or nearly three times as much as the rats fed standard bread (23 grams), average gains per week being 6 grams and 2 grams respectively. The rats fed extra yeast were also far superior in general physical appearance as can be seen from the pictures of rats 4 and 14 (fig. 4) that weighed the same at the beginning of the experiment and were fairly typical of their respective groups. Rats 3 and 13 (fig. 5) also weighed the same at the beginning of the study, and their pictures are reproduced for comparison.

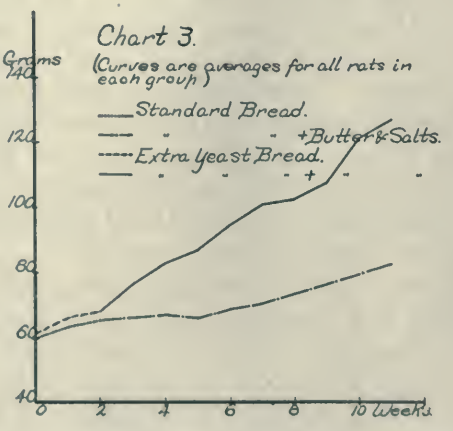
The bread used as a standard was by no means a poor quality bread but on the contrary was somewhat better than the average as the liquid used in its preparation was one-half milk, which is absent from some products. About 10 per cent of the protein of this bread was milk protein, while about one-fifth of the protein of the extra yeast bread was yeast protein, the latter also of course containing milk, to make a total of about 30 per cent of non-wheat protein.

Patent wheat flours and breads made from them without suitable addition are known to be deficient in water-soluble vitamine and in protein (9). The use of milk to the extent of one-half the liquid required or sufficient to replace 10 per cent of the wheat protein by milk protein did not lead to significant gains in weight, in spite of the higher quality of the proteins of milk. That the milk addition should

furnish sufficient water-soluble vitamine would not be expected from the relatively low vitamine content of milk as indicated by the work of Osborne and Mendel (10), who found even 15 cc. of fresh unpasteurized summer milk inferior to 0.2 gram of dried yeast in this respect.







The addition of 5 per cent of dried yeast to the flour, therefore, undoubtedly increased the content of water-soluble vitamine from a very low to an adequate level and also supplemented the protein of the flour in an important way. The fact that even with this 5 per cent yeast addition maximal growth was not attained indicates that a greater amount of yeast could be used to advantage in further improving the bread protein as well as for the stimulatory action of the yeast on

Rat 4

Rat 14

Fig. 4

Rat 3

Rat 13

Fig. 5

protein metabolism, which may be associated with the high vitamine content of yeast. Because of this high content of yeast in water-soluble B less of it would be required to make the bread adequate in this respect than to supplement properly its protein, and smaller amounts than would be necessary for the latter purpose would be of value in connection with diets containing moderate amounts of meat and milk, which would adequately supplement the bread protein, but which are low in water-soluble vitamine.

CONCLUSIONS

Flour containing 5 per cent of yeast powder makes a palatable bread much more nutritious than ordinary bread. The yeast supplements both the water-soluble B and protein content of wheat flour. Yeast is thus a nutrient constituent of bread, and any increase in its amount up to quantities far in excess of those ordinarily used will improve the food value of the product.

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THE EFFECT OF SUCKLING AND CASTRATION ON THE LACTATING MAMMARY GLAND IN RAT AND GUINEA PIG

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Received for publication January 14, 1921

According to Jentzen and Beuttner, Thomas Winn, an American farmer, first made the observation that in castrated cows milk production may continue for several years (1). Since then this observation has been frequently confirmed and the effect of castration on the composition of the milk studied. Marshall and Jolly moreover found that castration carried out during the latter part of pregnancy permits further growth of the mammary gland and subsequent lactation (2).

It has furthermore been observed that cows which are not milked "run dry," that evidently suckling acts as a stimulus of secretion (3). To our knowledge a systematic study of the course of lactation and the influence of suckling and castration on the mammary gland following labor has not yet been made. We used for our experiments the same rats and guinea pigs which served for the two preceding studies which concerned the uterus and ovaries in the period following labor.

THE MAMMARY GLAND IN NURSING RATS AND GUINEA PIGS

Six to twelve hours after labor the mammary glands in rat and guinea pig are in a similar condition. In both as a result of the proliferative processes during pregnancy the glands are large, but the epithelial cells have not yet reached their full size, neither are the acini as yet usually distended, although we find some acini with a larger lumen in the rat. A limited number of vacuoles indicate in both the onset of secretion.

There are, however, some differences between the guinea pig and rat. In accordance with the observations of Loeb and Hesselberg (4) we find in the guinea pig soon after labor a number of mitoses in acinus cells,

while they are quite rare in the rat. This corresponds to the effect of heat and ovulation on proliferative processes in the mammary gland of the guinea pig, which we described previously. Accordingly we find also a few mitoses in the duct epithelium of the guinea pig, but not of the rat. The condition of the stroma corresponds to the state of activity in the parenchyma. Thus we find in the guinea pig the connective tissue around the acini cellular consisting of fairly large fibroblasts with vesicular nuclei which show some mitoses, while in the rat the condition of the stroma is intermediate, the stroma consisting of thin strands of fibrous or fibrillar tissue with small cells and with a much smaller number of mitoses. In the rat, on the other hand, this growth period which in the guinea pig we called primary is absent.

Two days after labor we find in both rat and guinea pig further progress toward active secretion, but in the guinea pig the stage reached is somewhat further advanced than in the rat. In the rat the gland is red in color due to the presence of many distended capillaries, in the guinea pig the presence of milk gives it a reddish-white appearance. In both the gland epithelium has enlarged since labor, but in the guinea pig it has reached about the maximum size, while in the rat this stage has not quite been reached.

In both rat and guinea pig many gland cells contain vacuoles, indicating active secretion; but in the rat we find now in addition an active stage of mitotic proliferation in the gland cells which has apparently already passed in the guinea pig. In the rat we find accordingly also some mitoses in the gland ducts while they are absent in the guinea pig. In accordance with the activity in the parenchyma at this stage there is in the rat also more proliferation in the connective tissue cells than in the guinea pig. This proliferation is probably in some way connected with those conditions which lead to secretion. It is due to a stimulus which developed in the transition to the state of secretion in the gland cells rather than to the action of an ovarian hormone.

In both the stroma consists of thin fibers with small connective tissue cells. There is no marked difference between the castrated and non-castrated animals, but in the rat the number of mitoses is a little larger in the non-castrated animals, while the gland cells are of greater size in the castrated rats. In the stroma of the non-castrated guinea pig the number of mitoses is greater than in the castrated animal; these in all probability represent merely accidental variations.

There is, however, one noticeable difference between the rat and the guinea pig which remains constant throughout the period of lactation.

In the former amitotic proliferation of the nuclei is rare at all times, while in the guinea pig amitotically dividing nuclei are frequent. Amitoses and mitoses are to some extent mutually exclusive. We find therefore in the rat the number of amitoses as yet smaller than later in accordance with the fact that an active mitotic proliferation is going on at this period. Marked mitotic proliferation is furthermore incompatible with active secretion. Accordingly secretion has not yet reached full development in the rat at this period.

This proliferation in the mammary gland of the rat does not depend upon a substance given off by either corpus luteum or ovary as a whole, because it is present also in castrated animals, although not to so marked an extent as in non-castrated animals. The early proliferation in the guinea pig evidently depends upon the same factor that determines heat and ovulation.

In the guinea pig we observe in this specimen as also in later specimens a coalescence of some adjoining acini, the separating stroma being dissolved.

Seven days after labor secretion is fully established in the rat. Many acini contain large lumina filled with secretion. While the epithelium of the glands has reached its full height, in some of the larger acini the epithelium is flat probably as the result of the pressure of the increased secretion. Here also adjoining acini may coalesce. In accordance with the full establishment of secretion mitotic proliferation has now ceased and amitotic proliferation of the nuclei is somewhat more frequent, but in the rat amitoses remain on the whole rare. As in the guinea pig so also in the rat the higher epithelium of the glands,—and this applies especially to those cells in which the nuclei divide amitotically—the cytoplasm, takes on a bluish hemotoxylin stain. Around the acini the connective tissue forms a thin fibrous coat with small connective tissue cells which show very rare mitoses. There is no essential difference between the normal and castrated animal.

In the guinea pig conditions are similar to those found two days after labor. Secretion is fully established. Neighboring acini may fuse together. Some very dilated acini have flat epithelium as the effect of pressure. A few mitoses appear in the acini of the castrated guinea pig, and they are still somewhat more frequent in the ducts of the castrated animal. Mitoses happen thus in this case to be more frequent in the castrated than in the non-castrated guinea pig.

Two, three and four weeks after labor conditions are very similar in the mammary glands of rats to those found after one week. There exist

however some slight differences. In all of them fusion of neighboring acini may occasionally occur and this may lead to the formation of small cystlike formations.

While the gland epithelium is usually high in all of these animals, some more dilated acini have flat epithelium, the vacuolar part of the cytoplasm having already been discharged. At two and four weeks only exceptional mitoses are found, both in the castrated and non-castrated animals, and in parenchyma as well as in stroma. At three weeks mitoses are more frequent in the parenchyma (gland and duct cells) as well as in the stroma of the castrated animal. This mitotic proliferation must therefore be caused by conditions in the gland itself or its direct environment; it cannot be due to an ovarian hormone. On the whole the glands in the castrated and non-castrated animals behave exactly alike. There are always a few amitoses present in both. After three and four weeks a certain increase in the amount of red staining material is noticeable in gland and duct lumina. This may either indicate that the suckling has become less active at this time and that consequently a slight stagnation in the secreted material has occurred, or it may indicate that secretion is proceeding very actively, that consequently more material is produced than can be removed through suckling.

In the guinea pig conditions after two weeks are similar to those found after seven days. Occasionally neighboring acini fuse; some acini are more distended and lined with low cuboidal or flat epithelium. Mitoses are absent, but frequent amitoses are found. There is no difference between the non-castrated and the castrated animal. After three weeks some slight changes are noticed. At this period we are at or near the point where the transition from intense activity to a marked diminution in the nursing occurs. We find more colloid-like eosin staining material in gland lumina and ducts. Nuclear amitoses in gland cells are most frequent at this period and at some parts the epithelial cells are degenerating through vacuolization or pyknosis. We find a considerable hyperemia in both glands; some lymphocytes and polynuclear leucocytes are scattered in the stroma and occasionally small masses of leucocytes are found around and inside of some acini. These facts evidently indicate the presence of disturbing factors, perhaps due to a disproportion between secretion and removal of secreted material. It initiates the retrogressive changes. Exceptional mitoses are found in the parenchyma and stroma in the non-castrated animal. In all other respects the glands are like those found at two weeks. There is no difference between the castrated and the non-castrated animals.

The beginning of the retrogressive changes in the mammary gland of the guinea pig is much more marked after four weeks. At this period the guinea pigs no longer nurse their young ones to the same extent as before and the mammary gland is therefore smaller in both animals. The gland is still composed of many lobules, but they are much smaller than formerly and each lobule consists of many acini which are likewise now much smaller, although there are still some with a medium sized or large lumen. The gland cells also are smaller and lower; they are medium or low cuboidal. The number of vacuoles in the gland cells has much decreased and those present are small. The number of amitoses is likewise much smaller, but in some of the larger acini a few amitoses are still observed. The number of mitoses on the contrary has increased; we find about two or three mitoses in each section in the gland cells and also a certain number of them in the stroma, especially of the castrated animal. As usual a certain reciprocal relation exists between amitotic and mitotic activity, and again we find a certain parallelism between mitoses in parenchyma and stroma.

A direct destruction of gland tissue is indicated by the presence of karyolysis and karyorrhexis in some acinus cells. In accordance with all these findings the quantity of colloid-like material in the glands and ducts has much decreased.

While formerly only a thin fibrous coat surrounded the acini, we now find a large amount of dense fibrous tissue around the acini. Again some lymphocytes and polynuclear leucocytes are scattered in the stroma; some of the lymphocytes even are migrating through the epithelium of the acini and ducts and we find some masses of polynuclear leucocytes around and in some acini. This transitional stage evidently leads to disturbances which attract lymphocytes and polynuclear leucocytes. As we see, conditions are essentially similar in the castrated and the non-castrated animals.

After five weeks we find for the first time decided retrogression in the rat, while in the guinea pig, in which it had begun somewhat earlier, retrogression has made further progress at this time. In the rat the mammary gland has become much smaller; the lobules are smaller and each lobule consists of a much reduced number of acini which have a smaller lumen than formerly. The individual gland cells likewise are smaller. The number of vacuoles in the gland cells is reduced and there is less colloid-like material in the lumen of the acini and ducts. The connective tissue around the acini has now generally become densely fibrous and it is partly edematous. Those lobules which are surrounded by

dense connective tissue, usually consist of especially small acini; this may be due to some pressure exerted by the stroma. Some lymphocytes and polynuclear leucocytes are scattered in the stroma and some of them migrate through the glandular epithelium. In the castrated animal the mammary gland is similar.

We find therefore at five weeks in the rat conditions corresponding to those seen in the guinea pig after four weeks. There is a further similarity. Just as we found in the guinea pig with beginning retrogression at four weeks a decrease in amitoses and in other signs of secretion coinciding with a relative increase in mitoses, so we find also in the rat some increase in mitoses at five weeks; about two to three amitoses can be seen in the gland cells in each section. A few mitoses are also found in the ducts and in the stroma; again these mitoses are due to local changes and not to influence of an ovarian hormone.

In the guinea pig retrogression has still further progressed at five weeks. In both the castrated and the non-castrated animals the mammary gland has further decreased in size; the gland is still composed of many lobules, but they are now much smaller and each lobule consists of a smaller number of acini which latter are also generally very small. Some acini, however, are still somewhat larger. The epithelial cells are now lower and the number of vacuoles and also of amitoses has decreased considerably. They are observed in those acini which are still relatively larger. In the non-castrated animal we again find a relative increase in mitoses in the parenchyma as well as in the stroma, while in the castrated guinea pig mitotic proliferation has ceased at this time. The colloid-like material in acini and ducts is small in amount; the stroma is densely fibrous. In the non-castrated guinea pig the nuclei of the acinus cells are on the whole pale, while they take the stain well in the castrated animal. In both we find epithelial cells which degenerate by vacuolization, karyolysis or karyorrhexis; the degeneration is somewhat less pronounced in the castrated animal. In both guinea pigs many lymphocytes and some polynuclear leucocytes are found in the stroma and some of them migrate through the epithelium of acini and ducts, and collecting around and inside of acini, they may contribute to the destruction of the gland.

THE MAMMARY GLAND IN NON-NURSING RATS AND GUINEA PIGS

Two days after labor. In both rat and guinea pig the effect of the lack of nursing is noticeable as early as two days after labor.

In both the nursing and the non-nursing rat at this period the mammary glands are large, but while in the nursing animal they are red colored, indicating an active circulation, in the non-nursing rat they are pale. The lobules are somewhat smaller in size in the non-nursing rat. In the non-nursing animal the lobules consist of large acini, but this is mainly due to the considerable amount of vacuolar and colloid material found in the lumen of the acini and in the ducts. The ducts are dilated by the stagnating secretion. The gland cells themselves are here distinctly lower than in the nursing rat; they are low cuboidal or flat, although some cells are a little higher. Again we notice a coalescence of some neighboring acini. As in the nursing rats the gland cells contain vacuoles, which are more frequent in the non-castrated than in the castrated animals, but the nuclei are on the whole pale in the non-castrated rat, while in the castrated rat the nuclei stain well with hematoxylin. While in the nursing rats rare amitoses are found, they are lacking altogether in the non-nursing rats, indicating a cessation or diminution in secretory activity in the latter animals. On the other hand, a certain number of mitoses are observed in the gland cells of the non-castrated rat, they were exceptional in the castrated animal. In the nursing animals the stimulus of nursing evidently called forth a much greater mitotic proliferation. The character of the stroma is not yet changed, but the number of mitoses in the stroma is reduced in the non-nursing rats. There are as yet very few lymphocytes and polynuclear leucocytes in the stroma. With exception of the few differences mentioned the glands in the castrated and the non-castrated rats were similar.

On the whole we find analogous conditions in the nursing guinea pigs after two days. The glands differ here from those of the nursing guinea pigs in the following points: *a*, The gland cells are not quite so high; *b*, in the non-castrated animal the nuclei of the gland cells are pale; they stain well in the castrated animal; *c*, while in the nursing animals small vacuoles were seen at this period, in the non-nursing guinea pigs many epithelial cells contain granules. Some granules get into the lumen of the acini and some acini are filled with granules. *d*, in the non-nursing guinea pig some gland cells are degenerating (vacuolization, pyknosis, karyolysis, karyorrhexis); *e*, amitoses are frequent in the nursing, and very rare in the non-nursing animals; *f*, colloid-like material is more plentiful in the lumen of acini and ducts in the nursing than in the non-nursing animals; *g*, mitotic proliferation is rare in the parenchyma of the non-nursing animals, but a few mitoses are found while they are absent in the nursing guinea pig; *h*, in the non-nursing, non-cas-

trated guinea pig there is already a small increase in lymphocytes and polynuclear leucocytes in the stroma. This increase is lacking in the castrated animal.

Seven days after labor a great decrease in the size of the mammary gland has taken place in the rat, the diminution between the third and seventh day following labor is very marked. The gland is now of small size, while at two days it was still large; both the number of lobules and of acini is much diminished. There are only few lobules and few acini left in both the castrated and the non-castrated rats. The acini are of small size, they have a small lumen and low cuboidal epithelium which is also of small size. The absorption of the secreted material has much further progressed in the non-castrated animal; here very little colloid is visible in acini and ducts and all the ducts have a narrow lumen; there is more colloid-like material left in the acini and ducts in the castrated rat; here some of the ducts are even cystically dilated by retained secretion. Thus it comes about that in the castrated rat some acini appear larger than in the non-castrated rat. In some gland cells blood pigment can be seen. Only very few of the epithelial cells contain vacuoles. Evidently the back pressure of the secretion and the lack of the stimulating effect of the suckling cause a cessation in the formation and secretion of milk. As in all non-nursing rats amitoses are completely absent. On the other hand, in contradistinction to the nursing rats, there is still some mitotic proliferation going on in the gland cells of the non-nursing, non-castrated animal; it is absent in the castrated animal. The connective tissue has relatively increased around the acini; it is of a fibrous character, mitoses in the stroma are very rare.

In both the castrated and the non-castrated rats there are many lymphocytes and some polynuclear leucocytes present in the stroma; occasionally the polynuclear leucocytes form small collections. Some of these cells migrate through the glandular epithelium and thus they may injure some of the acini and ducts.

In the non-nursing guinea pigs the same process occurs at this period, but the retrogression is not so rapid as in the rat. As in the non-nursing rat the mammary gland is smaller than that of the non-nursing guinea pig two days after labor; it comprises still many, but much smaller lobules and each lobule has less acini which have a small or medium sized lumen. In the non-castrated, but not in the castrated guinea pig, there are occasionally some large acini present. The epithelium is low, cuboidal or flat in the acini of medium or small size, and somewhat higher and larger in some of the larger acini. It is therefore at places higher

in the non-castrated than in the castrated guinea pig. Some of the higher cells have large vacuoles and the nuclei divide here amitotically. There is therefore more vacuolization and more amitotic division in the non-castrated animal. There are only very few amitoses in the castrated guinea pig; but they are also much diminished in the non-nursing, non-castrated guinea pig as compared with the nursing, non-castrated guinea pig at the corresponding period.

The more active secretion in the non-castrated animal is also indicated by the greater amount of colloid-like material in the larger acini as well as ducts of this animal. The stroma is relatively more fibrous than at two days in the non-nursing animals and also as compared to the nursing guinea pigs; it is especially dense around the smaller acini. The connective tissue nuclei are small.

In contradistinction to the amitoses, we find a considerable number of mitoses mainly in the gland cells, less in the gland ducts. The mitoses in the connective tissue cells are likewise much increased. In the nursing guinea pigs mitoses are lacking or very rare at this time. In a similar way we find an increase in the number of mitoses in the non-nursing rat at this period, but in the rat it is still more marked in the following period.

In the non-castrated guinea pig many lymphocytes and some polynuclear leucocytes are found in the stroma and some of them migrate through the epithelium of the gland and destroy some parts of it. In the castrated animals the number of lymphocytes is much smaller; again they injure here some of the acini.

In contradistinction to the rat we again find in the guinea pig marked degenerative processes in the gland tissue. Vacuolization, karyolysis, pyknosis and karyorrhexis are seen in the gland cells. Lymphocytes may participate in this destruction. At some places the epithelium has disappeared, and the colloid material is entering the stroma, and connective tissue begins to proliferate and to fill spaces formerly occupied by gland tissue. In the castrated guinea pig the amount of degeneration is less.

Two weeks (15 days) after labor. In the rat the size of the gland has still further decreased and has almost reached its lowest level. It consists of very few and small lobules and isolated ducts. Each lobule comprises very few small acini. The gland cells are small and only a few of them contain vacuoles. Little colloid-like material is found in acini and in the ducts, which latter have a very narrow lumen. The stroma is fibrous, but small in amount; it contains some lymphocytes

and polynuclear leucocytes, some of which migrate through the epithelium of acini and ducts. There is a considerable mitotic proliferation going on in the glands of the castrated and the non-castrated animals; it is more marked in the latter. The mitoses in the stroma are exceptional. In the castrated rat the lumina of the acini are furthermore smaller and they contain less secreted material than in the non-castrated animal. There are also fewer vacuoles present in the castrated rat. Otherwise both behave alike.

In the guinea pig a more steady, less abrupt decline in the size and activity of the mammary gland takes place; the gland is small and contains fewer lobules and fewer acini than formerly. In general the acini are small and the gland cells are low cuboidal and also of small size; however, around the larger ducts there are some acini with a large lumen and lined by high cuboidal epithelial cells. It is especially these higher cells which contain vacuoles and divide amitotically or degenerate in the usual manner. On the whole the occurrence of vacuoles and amitoses in the gland cells is infrequent. Some products of secretion are seen in the large acini and in the ducts. The stroma is fibrous; there is more dense fibrous tissue around the small than around the larger acini.

There are many lymphocytes and some polynuclear lymphocytes in the stroma and some of them are passing through the epithelium and destroying it. Mitoses are quite frequent; but they are more frequent in the non-castrated than in the castrated animals; in the former they are also frequent in the stroma. In the castrated guinea pig not only the mitoses are less, but the high gland cells are missing; there are furthermore fewer amitoses, fewer degenerating cells and a smaller number of lymphocytes and polynuclear leucocytes.

After three weeks the mammary gland in the non-nursing rat is similar to that found after two weeks. Glands and acinus cells are small. At this time as after two weeks some blood pigment can be found in the epithelial cells. The ducts are mostly isolated, unaccompanied by acini. Some masses of lymphocytes and polynuclear leucocytes are encountered around some acini and some of the lymphocytes migrate through the glandular epithelium.

The number of mitoses in the gland tissue proper happens to be much reduced in this specimen; they are lacking altogether in the stroma. The gland of the castrated animal differs from that of the non-castrated rat in the following points: In the former the acini and the gland cells are somewhat smaller; there are less vacuoles, less mitoses, less products of secretion; furthermore the number of lymphocytes and polynuclears is smaller.

In the guinea pig also the glands are on the whole similar to those found after two days and we notice the same differences between the glands of the non-castrated and the castrated animals as before. However, large acini are now absent and degenerating cells are no longer found; amitoses are still rarer than before. Mitoses are not infrequent; they occur in both parenchyma and stroma. While quite a number occur in the gland cells in the castrated guinea pig they are here not so frequent as in the non-castrated animal. In the castrated guinea pig the mammary gland as a whole is smaller and the individual lobules and acini are also smaller. The acini lined with larger and higher epithelial cells are here not visible. The number of vacuoles, and the quantity of secreted material in the acini and the number of lymphocytes in the stroma is less; but even in the castrated animal we find some masses of lymphocytes and polynuclear leucocytes around and inside some acini.

Four weeks after labor the mammary gland in the non-castrated, non-nursing rat is small, but perhaps not quite so small as at three weeks; the number of lobules and acini is slightly larger. The main difference, however, consists in the very much larger number of mitoses which we find here everywhere in acinus, duct cells and stroma. Some ducts have still a wide lumen. Some acinus cells and connective tissue cells contain blood pigment.

Where there existed in the previous periods some difference between the non-castrated and the castrated non-nursing rats, it is still more marked in these specimens. In the castrated animal the gland is smaller, consists of fewer lobules and acini which latter are very small; the gland cells themselves are also much smaller. The ducts have a narrow lumen and the amount of secreted material is less. The number of mitoses is very much less in all the structures. There are here fewer lymphocytes and polynuclear leucocytes in the stroma than in the non-castrated animal.

In the guinea pig at this time no marked change has taken place from the preceding period except that the number of mitoses has decreased very much in both the non-castrated and the castrated animals; they are very rare in the non-castrated and absent in the castrated animals, The number of vacuoles and amitoses in both animals is very small. There is a very small quantity of thick colloid-like material in some acini and in the ducts. Again there are in the non-castrated animal many lymphocytes and polynuclear leucocytes present and some of them migrate through the gland epithelium; occasionally large collec-

tions of the lymphocytes are observed around and in some acini which are partly destroyed. In the castrated animal the number of lymphocytes and polynuclear lymphocytes present is smaller.

After five weeks the mammary gland in both the castrated and the non-castrated rat is very small and hardly visible to the naked eye. It is composed of very few lobules and isolated ducts. In each lobule there are very few and very small acini which are lined with small low cuboidal epithelium. Mitoses are now exceptional in the glands of both the castrated and the non-castrated rat. Colloid-like material is very small in amount or absent. Very few acinus cells have vacuoles and they are fine. The ducts are very narrow. The stroma around the acini consists of little fibrous connective tissue, with small cells. Some epithelial and stroma cells contain blood pigment. There are some lymphocytes and polynuclear leucocytes in the stroma and some of the lymphocytes are passing through the gland epithelium.

In the castrated animal the acinus cells are somewhat smaller and there are fewer lymphocytes and polynuclear leucocytes present in the stroma; otherwise both glands are similar. At this time the lowest point in both size and activity of the glands has approximately been reached in the glands of the castrated and the non-castrated rats.

In the non-nursing guinea pigs the mammary gland also shows a still further decrease in size from the former period. It consists now of a few lobules and a few small acini; the acinus cells are small and low. Only exceptional mitoses and very rare mitoses are visible. The number of vacuoles and the amount of colloid-like material is very small. The stroma is fibrous and partly slightly edematous. There are many lymphocytes and some polynuclear leucocytes and some of them collect around the glandular epithelium and migrate through it. In the castrated guinea pig the number of lymphocytes is smaller.

We see then that at five weeks in both non-nursing rat and guinea pig the lowest state has been reached and that at this time the difference between the mammary gland of the castrated and the non-castrated animal has almost disappeared.

DISCUSSION

1. Curves of activity and size of the mammary gland in the nursing animals. In both rat and guinea pig the mammary gland has not yet reached its maximum secretory activity ten or twelve hours after labor. This is attained in the guinea pig two days and in the rat about seven

days after labor, or somewhat earlier; the maximum is therefore reached earlier in the guinea pig. From this period on the gland maintains approximately the same condition for several weeks. In the rat a maximum of secretion is found three and four weeks after labor; then a sudden diminution in the size of the glands and of the gland cells is noticeable five weeks after labor. This goes hand in hand with a diminution in those signs which indicate secretory activity in the gland. In the guinea pig a critical point is reached three weeks after labor, when we find associated with certain conditions indicating a maximum of secretion (maximum of amitoses, much colloid material) the first signs of degeneration in some gland cells. In the following two weeks we find involutionary changes, which are much more marked at five than at four weeks after labor. In the guinea pig in which the young at the time of birth are much more mature than in the rat, suckling ceases at an earlier period.

Five weeks after labor we find in the rat a marked decrease in the size of lobules and acini, in the number of acini and in the size of the gland cells; in the guinea pig a similar decrease occurs four and five weeks after labor.

2. Besides changes in the size of the lobules and acini and in the size and shape of cells, we find changes in the processes of secretion, namely, in the number and size of vacuoles, the frequency of amitoses in the acinus cells and the amount of colloid-like material in the lumen of the acini and ducts.

In the rat the number of vacuoles increases in the first two days after labor; a decrease occurs at five weeks. In the guinea pig the initial increase is simultaneous with that in the rat; the decrease in number and size of vacuoles is very marked at four weeks and still more pronounced at five weeks.

Amitoses occur in the rat as well as in the guinea pig when the gland cells have reached a large size; but throughout they are much more frequent in the guinea pig than in the rat. They decrease in rat and guinea pig with the cessation of suckling. The diminution in the colloid-like material at this period is especially noticeable in the guinea pig.

3. The mitotic proliferation in the glands shows an interesting relation to the activity or cessation of activity in the glands. In the rat an active mitotic proliferation in the parenchyma and stroma is found two days after labor; this coincides with the peroid of ascent toward full secretory activity. At that time the number of amitoses in the

rat has not yet reached a maximum. When the secretory activity is fully established mitotic activity is very low; mitoses are exceptional and occasionally a somewhat increased number occurs, as for instance three weeks after labor in the castrated rat. But with the cessation of secretion five weeks after labor, at a time when the number of amitoses is much diminished, the mitotic activity has again become quite active.

In the guinea pig we find a marked mitotic activity soon after labor, at a time when a new ovulation is imminent and the animal is in heat. With the full development of secretion and of amitotic activity mitoses become very rare in parenchyma and stroma; but as in the rat with beginning cessation of secretion and diminution in the number of amitoses the mitoses become again frequent after four weeks; this mitotic activity is still present but with diminished intensity five weeks after labor. On the whole mitoses in the parenchyma and in the stroma follow a parallel course. As a rule an increase in the number of mitoses in the stroma is found concomitantly with an increase in the parenchyma; but occasionally mitoses are increased in the stroma without a simultaneous increase in the parenchyma and vice versa. It is possible that sometimes the increase in the mitotic activity of the stroma has just passed or not yet begun at the time of examination. On the whole this parallelism to which we had drawn attention previously is unmistakable.

This appearance of what might be designated as alternating proliferating activity in rat and guinea pig does not depend on hormones secreted by the gonads; it occurs also in castrated animals. It is in the main due directly to changes in the mammary gland itself.

4. While in the actively proliferating mammary gland of the guinea pig the stroma is at least in parts rich in fibroblasts, in the secreting gland of the nursing animal the stroma is fibrous in the guinea pig as well as in the rat. However, the amount of fibrous tissue around the secreting acini is very small. As soon as the secretion ceases, the amount of fibrous tissue around the acini increases considerably. This is perhaps to a great extent due to a retraction of the fibrous stroma which has been made possible through the diminution in the size and number of the acini.

5. Lymphocytes and polynuclear leucocytes are found primarily in the stroma. Secondarily they often collect around certain acini and penetrate through the acini or lining of the ducts into the lumen of the gland. In the rat as well as in the guinea pig they appear when the secretion begins to cease and an involution of the gland sets in. Thus

we find them in the rat five weeks after labor; in the guinea pig they appear earlier, namely, three weeks after labor; they are visible four weeks after labor and become still more numerous at five weeks. A small number of them is seen in the guinea pig soon after labor and in the beginning of secretion. Somehow the changes produced by cessation of secretion attract these cells. They are on the whole less numerous in the castrated animals. This is especially noticeable in the series of non-nursing animals; but it is likewise evident in the series of nursing animals five weeks after labor.

6. The mammary glands in the castrated and the non-castrated rats and guinea pigs are essentially alike during the period of secretion. The effect exerted by the ovary is negligible in comparison with the effect exerted by the suckling and with the changes brought about through the sudden cessation of growth stimuli in a mammary gland in which hyperplasia had been previously caused through the growth factors active during pregnancy.

There are minor differences between the castrated and the non-castrated animals; they are probably of an accidental character, but our findings in the non-nursing animals where the differences are more definite suggest that also in the first series certain differences between the castrated and the non-castrated animals may be due to the influence of the ovary. They are the following: *a*, Two days after labor the number of mitoses is greater in the mammary gland of the non-castrated than of the castrated rat. In a similar way the number of mitoses is much greater in the mammary gland of the non-castrated than of the castrated guinea pig five weeks after labor. We interpret these facts as indicating that the effect of the ovarian hormone may be effective in the time following secretion or preceding it. *b*, In the rat the number of lymphocytes in the stroma of the mammary gland five weeks after labor is less in the castrated rat than in the non-castrated rat. This difference between the castrated and the non-castrated animals is more pronounced in the series of non-nursing animals.

7. It might be asked whether the growth stimuli which cause the growth of the mammary gland in the normal pregnant guinea pig might be equally efficient in causing proliferation in the gland of the guinea pig, nursing her young in the period following labor. In our previous paper we gave some facts which make it very probable that in the suckling guinea pig these growth stimuli of pregnancy are ineffective (4). We found that fifteen, nineteen and twenty-nine days after labor in guinea pigs which had been impregnated directly following

labor and which suckled their young, mitotic proliferation was absent in the glands, that on the other hand the typical amitotic proliferation of the nuclei was in evidence.

We may then conclude that the processes active during secretion successfully counteract the influence of the growth stimuli of pregnancy.

8. The curves of involution of the mammary gland in the non-nursing animals take a somewhat different course in the rat and in the guinea pig. In the rat the descent is very rapid; it is already noticeable after two days, becomes very marked after seven days, and has almost reached its lowest point at fifteen days following labor; it progresses extremely slowly during the following four weeks. Lobules and acini decrease in number and size; epithelial cells become smaller. In the guinea pig the main decline takes place also in the first two weeks, but the decrease is somewhat less abrupt and proceeds more evenly toward end of the fifth week. The essential changes involved in involution are in both species the same. This difference in the curves is perhaps referable to the fact that in the rat the gland tissue is distributed over a much wider area than in the guinea pig where the gland is concentrated in two places. It is of interest to compare these curves with those of the retrogressing uterus. Here we also find a more rapid decline in the rat than in the guinea pig.

9. The changes found in the non-nursing animals are the same as those found in the nursing animals after cessation of suckling, namely, besides the diminution in size and number of lobules and acini a diminution in the number of vacuoles, in the size of the acinus cells, in the amount of colloid-like material in acini and ducts, in the character of the stroma, which becomes more densely fibrous and is present in a relatively larger quantity in the nursing animals. The number of nuclear amitoses is much diminished in the guinea pig; they are absent in the non-nursing rat.

There is an additional difference between rat and guinea pig; in the latter degenerative processes in the gland cells are much more marked than in the rat. In the guinea pig gland cells degenerate under the appearances of pyknosis, karyorrhexis and karyolysis and cytoplasmic vacuolization in the first two weeks following labor; a maximum in these changes is reached about one week after labor. At that time whole parts of the gland may be substituted by connective tissue. In the rat these degenerative processes are not noticeable. This difference is perhaps again due to the fact that in the guinea pig the whole gland tissue is concentrated in two areas; therefore the destructive processes are here more violent than in the rat.

10. We found in the series of nursing animals a migration of lymphocytes and polynuclear leucocytes from the vessels into the stroma of the gland and from these into the acini and ducts. The same condition we find in a more marked manner in the non-nursing series. Here we find throughout an emigration of lymphocytes and polynuclear leucocytes. It increases in the first few days following labor and is fully developed seven days after labor. In the guinea pig it is at all times much stronger in the non-castrated than in the castrated animals; in the rat the same difference becomes apparent from the third week on.

The substances produced in the gland during the process of involution attract these cells. These processes of involution are therefore more marked in the non-castrated than in the castrated animals.

11. A study of mitotic proliferation in the non-nursing series confirms the conclusions we arrived at in the analysis of the glands of the first series. Here we found that cessation of nursing was accompanied by a resumption of mitotic proliferation in the formerly secreting glands. In the non-nursing rats we note active mitotic proliferation two, seven and fifteen days after labor. At three weeks proliferation is suspended; but at four weeks it is once more active, reaching here a maximum in the non-castrated animal. In the non-nursing guinea pig mitotic proliferation is very active seven days after labor, it is still quite noticeable, although less intense two and three weeks after labor. Four and five weeks after labor it has again become rare. There is in general a correspondence between mitotic activity in the stroma and parenchyma. In the rat we find throughout the non-nursing series and in the guinea pig two and three weeks after labor, a decided inferiority of the castrated animals, as far as mitotic proliferation is concerned. But even in the castrated animals there is present an active endogenous stimulus for cell proliferation which is independent of an ovarian hormone. It is evidently some factor active in the gland itself which at certain times causes this mitotic cell proliferation. This stimulus is probably at least at a certain period identical with the stimulus which calls forth the secretion of milk. At other times it is probably related to those regressive conditions in the parenchyma which follow milk secretion, when we find degenerative processes in the gland or solution of gland tissue. Both of these conditions are of a similar character; in both we have to deal with degenerative processes and with a discarding either of a part of the cytoplasm or of the whole cell. In a certain sense they may therefore be classed among the regenerative stimuli. In the secretory gland these stimuli merely lead to an amitotic division of the nuclei;

but as soon as secretion ceases mitotic proliferation can set in. We find therefore here an alternating relation between amitotic and mitotic proliferation.

12. There is present in addition to the local proliferative stimuli a secondary condition emanating from the ovary which reinforces the action of the local stimuli. Thus we find that in the castrated animals mitotic proliferation is less marked than in the non-castrated animals, although at the time when the endogenous stimuli are at the height of their action proliferation may reach the same intensity in the castrated and non-castrated animals.

13. We have stated that in the castrated animals mitotic proliferation in the mammary gland was less than in the non-castrated rats and guinea pigs. There was furthermore some difference in the amount of infiltration with lymphocytes and polynuclear leucocytes in these two kinds of animals. A comparison of the glands in the non-nursing series shows still further differences between the castrated and the non-castrated animals. In the rat the size of acini, of acinus cells, the number of vacuoles and of the amount of colloid are usually somewhat smaller in the castrated animals. In the guinea pig we find similar differences in the size of the acinus cells and in the number of amitoses in the first three weeks following labor in the non-nursing series.

We may then conclude that the effect of the ovarian hormone on the mammary gland is present even in the period following labor, that its effect is however covered up by the much stronger effect of suckling and that as soon as this stimulus ceases the effect of castration becomes again apparent, although it may be weak.

14. It has been observed that the suckling of the breast on one side may cause the pressing out of milk from the breast of the other side (5). This suggests the participation of reflexes which connect the separate glands in the process of suckling. It was therefore of interest to test the effect of suckling in one breast on the involutionary changes in the other breast, which take place if here suckling has been made impossible through ligating the nipple. This we did in several cases in nursing guinea pigs. In each case we found that the gland which could no longer be used for suckling retrogressed rapidly, in a way comparable to the involution of the mammary gland in the non-nursing guinea pig; on the contrary the gland of the other side which was suckled by the young continued to be large and to produce milk. We may therefore conclude that the process which maintains the active state of lactation in the mammary gland consists solely in the local effect of the withdrawal of the milk.

15. Since Virchow it is customary to distinguish between functional and formative stimuli. The latter lead to multiplication of cells and nuclei, to growth generally and to differentiation, the former initiate those changes which lead to function unaccompanied by growth. The stimuli initiating lactation are of an intermediate character; they produce a combination of processes of secretion and of an abortive growth, usually consisting in amitotic nuclear division, but under certain conditions advancing to mitotic cell proliferation.

In the case of the mammary gland the processes of secretion probably involve a loss of parts of the cytoplasm and it is probably this factor which is responsible for phenomena of growth which we observe during lactation or following it.

SUMMARY

1. Castration does not noticeably modify the condition of the lactating mammary gland in rat and guinea pig. The effects of castration become manifest as soon as suckling ceases.

2. The changes found in the mammary gland during lactation, after cessation of suckling and in animals which have been prevented from suckling are similar in rat and guinea pig, but some differences exist in the time curves in both species in nursing as well as in non-nursing animals; differences exist furthermore in the frequency of amitotic proliferation, and the kind and intensity of degeneration of gland tissue in both species.

3. An alternating relation exists between mitotic cell proliferation and amitotic nuclear proliferation in the mammary gland. The latter is accompanying secretion, the former precedes and follows secretion. Both are associated in a causal way with the cellular processes on which lactation in all probability depends (a partial solution of cytoplasm) or which initiate the return of the gland to the resting condition (total solution of parts of tissue).

4. The stimulus calling forth lactation is intermediate in character between a functional and a formative stimulus and partakes somewhat of the character of both.

5. The stimuli which call forth proliferation in the mammary gland during lactation and directly following lactation are essentially of an endogenous character; there is secondarily added to this stimulus the action of ovarian hormone. Lactation prevents the action of this hormone from becoming manifest. It manifests itself with the cessation of lactation.

6. Secretion not only prevents the ovarian hormone from manifesting itself in the mammary gland, but also those formative stimuli which are active during pregnancy.

7. Involution of the mammary gland in the non-suckling animal is essentially a local process; it is induced in the mammary gland by the lack of use in one particular gland, without reference to the function of other glands.

8. Invasion of the mammary gland by polynuclear leucocytes and lymphocytes occurs especially during the period of involution in contradistinction to that of lactation. It is less marked in the castrated animals.

9. The stroma of the secretory gland consists of thin connective tissue fibers, in contrast with the proliferating gland where the stroma is often cellular. In the resting gland the stroma is densely fibrous.

10. If we compare the influence of castration and nursing on involution in uterus and mammary gland, we notice in the mammary gland a dominance of the effect of nursing over that of castration, while in the uterus the opposite relation exists.

In both species our results show a very rapid effect of castration and nursing on the activities and size in both organs and especially do they indicate a very early diminution in proliferative activities of the tissues as the result of castration.

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DIET AND SEX AS FACTORS IN THE CREATINURIA OF MAN

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Received for publication January 17, 1921

The cause of the excretion of creatine by women is as yet unexplained. Krause (1) observed creatinuria regularly immediately following menstruation and considered that the excretion was related to changes associated with the sexual cycle in woman. M. S. Rose (2) and W. C. Rose (3) and collaborators, however, were not able to confirm the work of Krause. They found the creatinuria irregular and without any relation to the period of menstruation. Great variations between individual subjects were also observed. Krause (4) has suggested that women occupy a position intermediate between men and children as far as concerns their creatine metabolism. Men are able to completely metabolize the creatine normally produced in metabolism; in children, the power of conversion or of destruction of creatine is very slight; while according to the conception of Krause, women are able to metabolize the greater part, but frequently not all, of the creatine which is normally the product of the metabolic activities of the muscles. This implies that there is a diminished power of destruction of creatine in women, but as far as is known to the writers, this is based on indirect evidence and has never been demonstrated experimentally. Denis and Minot (5), (6) have recently maintained that the excretion of creatine by women is associated with a high level of protein metabolism, since even in normal women diets high in protein caused the excretion of large amounts of creatine. They had previously shown that in adults of both sexes in cases of Graves' disease (7) and in normal children (8) the output of creatine varied with the level of protein metabolism. With normal men however creatinuria could not be induced by ingestion of excessive amounts of protein. The theory of Miss Denis points to an exogenous origin of creatine from protein and to a definite saturation point of the organism for creatine, beyond which excretion of creatine whether exogenous or endogenous takes place. The muscles of women and children even allowing for individual

variations, would be assumed to have a lower saturation point than those of men since creatinuria may be so readily induced by increases in protein content of the diet in the former. Several investigators (2), (3) have recently reported that they have been unable to confirm the work of Denis and Minot. Denis and Minot (6) however maintain that the diets employed by W. C. Rose (3) were not sufficiently high in protein content to cause creatinuria. It is to be noted that in the earlier experiments of Denis (5), creatinuria was obtained at essentially the same level of nitrogen excretion as was reached in the experiments of Rose. The criticism is also made that the periods of high protein diet followed a low protein diet and were too brief to give opportunity for the production of creatinuria. In three of the four high protein periods with normal women reported by Miss Denis (5), (6), however, creatine appears on the first day of the high protein period despite a previous diet of low protein content.

The experiments of the present series were undertaken to afford additional evidence on the following points since the experimental data of previous workers seemed conflicting. *a*, The relation of creatinuria to the sexual cycle in the normal adult female. *b*, The influence of the protein content of the diet on the creatinuria of women. *c*, The ability of the female organism to destroy creatine administered per os. Is the destruction or transformation of ingested creatine by the adult female influenced by variations in the sexual functions?

METHODS

Creatinine was determined by the microchemical method of Folin, creatine by the method of S. R. Benedict, pure creatinine being used as the standard in both cases, and total nitrogen by the method of Kjeldahl-Gunning.

Three healthy women, students or instructors in the laboratory, served as subjects of the experiments. They were all maintained on a creatine-creatinine free diet during the periods of study. Milk and foods in which milk was present to any great extent were also excluded from the diet. The low protein diet consisted chiefly of potatoes supplemented by bread, butter, fruit and green vegetables. The high protein diets of the earlier experiments consisted mainly of eggs, beans and bread, while later the protein was supplied by glidine, a commercial diabetic food of high nitrogen content, supplemented by legumes and cheese. The food was not weighed, but the intake was

relatively constant in the periods of low protein intake. In the periods of high protein ingestion, however, the consumption of food was not uniform, an attempt being made to secure a maximum

TABLE 1

DATE	SUBJECT S., WEIGHT 56 KGM.			SUBJECT A., WEIGHT 57 KGM.		
	Creatinine	Creatine as creatinine	Total N	Creatinine	Creatine as Creatinine	Total N
1919	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
May 7	1.07	0.0	4.62	1.08	0.0	4.90
8	0.84	0.18	6.72	0.75†	0.0	3.20
9	1.10	0.0	6.00	1.04	0.0	6.70
10	1.18*	0.0	5.81	1.04‡	0.14	5.26
11	0.84*	0.0	4.18	0.84‡	0.10	3.56
12	1.16*	0.0	7.25	1.31‡	0.0	6.82
13	1.04*	0.0	5.50			
14	0.75	0.32	5.74			
15	1.10	0.0	6.50	0.94‡	0.27	6.26
16	0.98	0.16	7.58	0.90‡	0.0	6.97
17	1.08	0.10	5.94	1.04‡	0.0	5.10
18	1.08	0.0	5.09	1.08‡	0.05	5.97
19	0.97	0.04	6.31	1.04	0.17	6.85
20	1.02	0.0	5.60	1.04	0.0	5.90
21	1.10	0.0	5.70	1.04	0.0	5.60
22	0.80	0.0	5.80	1.04	0.3	5.85
23	1.03	0.0	7.22	1.16	0.0	5.18
24	1.03	0.09	9.59	1.16	0.0	6.82
25	1.02	0.06	9.66	1.02*	0.0	4.77
26	1.02	0.13	12.27	1.13*	0.0	4.05
27	1.12	0.0	13.46	1.13*	0.0	6.77
28	0.98	0.0	13.82	1.02*	0.06	5.70
29	1.12	0.0	13.85	1.10	0.0	6.40
30	1.12	0.0	12.90	1.02	0.11	7.15
31				0.98	0.04	7.11
June 1				1.02	0.05	8.63
2				1.07	0.05	6.60
3				1.14	0.07	10.56
4				1.08	0.0	7.37

* Menstrual period.
† Incomplete collection.
‡ Illness, overstrain.

rather than a uniform excretion of nitrogen. The experiments recorded in table 1 were carried out at the close of an unusually difficult year of work incident to the confused conditions of the year

1918–19. A comparison of the results of these experiments with those of later ones in which the same individuals served as subjects shows rather striking differences in the frequency of the occurrence of creatine in the urine. In the earlier experiments warm weather made the continuance of the high protein diets difficult, so that later experiments were more successful from this point of view.

DISCUSSION OF RESULTS

Relation of the excretion of creatine to the menstrual period. According to Krause (1), creatine normally disappears from the urine at the onset of menstruation, reappearing immediately after menstruation has ceased. His experiments are open to the objection that the analyses were made at irregular intervals sometimes a month apart. The results given in the tables of the present study afford data from three women under carefully controlled conditions and include eight complete menstrual periods with fore and after periods. In table 2 are presented data covering two successive menstrual periods with the whole of the intervening period. For one subject (S) the data include five complete menstrual periods.

The findings may be briefly summarized as follows. In four cases creatine was present just before the onset of the menses; in four (leaving out of consideration those days in which creatine was ingested), it occurred during the menstrual period; and in three, creatinuria was observed shortly after menstruation. In no case was a tendency toward creatinuria more marked during or near the period of menstruation than at any other time. In fact except in the first two experiments (table 1), which will be discussed later, creatinuria was not only rare and of very brief duration when it occurred, but the amounts excreted were very small. The results seem to justify the conclusion that the periodic variations in the sexual function in women can bear no direct relation to the intermittent creatinuria frequently observed, a conclusion in harmony with more recent work (2), (3). In this connection it is of interest to recall that although according to Hunter and Campbell (9) the total creatine content of the blood of women is higher than that of men, no variation in the content of the creatine of blood during menstruation has been observed by Wang and Dentler (10). The analyses presented however include only one menstrual and one intermenstrual sample for each individual. The value of their results would be greatly increased if analyses had been made at more

TABLE 2

DATE	SUBJECT S., WEIGHT 56 KGM.		
	Creatinine	Creatine as creatinine	Total N
1919	*grams*	*grams*	*grams*
June 26	1.03	0.00	5.89
27	1.04	0.00	5.18
28	0.24†	0.00	
29*	0.49†	0.00	
30*	0.90	0.04	4.55
July 1*	0.90	0.00	4.94
2*	**1.08‡**	**0.55**	**6.43**
3*	1.08	0.06	6.33
4	1.10	0.00	6.21
5	1.12	0.00	6.71
6	0.94	0.00	5.90
7	**0.99§**	**0.00**	**5.23**
8	1.05	0.00	5.20
9	1.07	0.00	5.10
10	0.95	0.00	4.40
11	0.97	0.00	5.10
12	1.07	0.00	5.66
13	0.89	0.00	5.43
14	1.07	0.00	5.33
15	1.07	0.00	4.83
16	1.05	0.00	6.00
17	1.03	0.00	5.45
18	1.07	0.00	5.20
19	1.11	0.00	5.18
20	0.92	0.00	4.52
21	1.03	0.00	5.08
22	**1.39****	**0.00**	**4.92**
23*	1.07	0.12	5.66
24*	0.92	0.00	5.02
25*	**1.07††**	**0.00**	**4.78**
26*	1.01	0.00	5.15
27*	1.01	0.09	5.88
28*	1.04	0.00	5.44
29	1.05	0.00	4.90
30	1.04	0.00	5.27

* Menstrual period.
† Incomplete collection.
‡ 0.494 gram creatine per os.
§ 0.502 gram creatine per os.
** 0.498 gram creatinine per os.
†† 0.500 gram creatine per os.

frequent intervals on the same individual and checked by urinary creatine determinations, in view of the relation observed by Hunter and Campbell between the creatine content of blood and urine.

Protein ingestion and creatinuria. The failure of other investigators (2), (3) to produce creatinuria in normal women by high protein diets as had Denis (5), (6) suggested that there might be a relation between the menstrual cycle and creatinuria following protein ingestion. The organism might at some period of the menstrual cycle be more easily stimulated to creatinuria by ingestion of excessive quantities of protein than at others. Denis does not indicate in her experiments the relation of the protein periods to the period of menstruation. Accordingly in the present study of the influence of high protein diets an attempt was made to determine whether there was any difference in the influence of the protein at different periods of the menstrual cycle.

In the case of subject S (table 1), the ingestion of larger amounts of protein seemed at first to give rise to creatinuria, but even with continued increase in the urinary nitrogen, creatine disappeared entirely from the urine, suggesting that the metabolic processes may be able to adjust themselves to the higher protein level. With subject A, although the increase in the nitrogen output was not as marked, creatine appeared persistently. This may possibly be attributed to the fact that the subject was suffering at the time from a mild attack of hyperthyroidism (7). In later experiments with the same subject when the thyroid condition was improved (table 4) no creatine excretion was observed to accompany a higher protein level. With subject S at a later period (table 3) creatine appeared only once although the nitrogen excretion was three times as great in the high protein period as in the preceding low protein one. The results indicate that in these subjects, at least, there is no more tendency to creatinuria during menstruation when the diet is high in its content of protein than when it is low; in fact in table 3 during the high protein period there was a total absence of creatine in the urine during the menses. Creatinuria was no more easily provoked by a high protein diet in one period of the menstrual cycle than in another. The failure to induce significant creatinuria by high protein diets is in accord with the findings of M. S. Rose (2) and W. C. Rose (3).

Gamble and Goldschmidt (11) have recently demonstrated that the creatinuria of infants associated with high protein diets derived largely from milk is not due to the protein per se, but probably to the creatine content of the milk ingested. They observed that the quantity

TABLE 3

DATE	SUBJECT S., WEIGHT 56.7 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. Low protein diet			
1919	*grams*	*grams*	*grams*
October 24	1.15	0.14	7.55
25	0.97	0.00	5.18
26	1.06	0.00	6.96
27	1.08	0.00	5.45
28	1.02	0.00	6.27
29	1.02	0.00	5.71
30	1.12	0.00	7.09
31			
November 1	1.12	0.00	5.88
2	0.96	0.00	5.43
3	1.02	0.00	5.62
4	1.04	0.00	5.55
5	1.08	0.00	
Period II. High protein diet			
6	1.04	0.00	7.37
7*	1.08	0.00	15.56
8*	0.93	0.00	16.75
9*	1.07	0.00	13.57
10*	1.06	0.00	16.50
11	0.97	0.00	14.26
12	0.99	0.02	20.34
13	0.96	0.06	16.81
14	1.16	0.00	19.60
15	1.04	0.00	14.74
16	0.99	0.00	10.46
17	0.95	0.00	11.20
Period III. Low protein diet			
18	**1.06†**	**0.02**	**12.68**
19	1.05	0.00	10.52
20	**1.15‡**	**0.03**	**8.76**
21	1.17	0.00	8.25

* Menstrual period.

† 1.0 gram creatine per os.

‡ 1.0 gram creatine per os in divided doses.

TABLE 4

DATE	SUBJECT A., WEIGHT 65.0 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. Low protein diet			
1919	*grams*	*grams*	*grams*
October 25	1.19	0.0	5.71
26	1.06	0.11	6.99
27	1.24	0.0	6.04
28	1.16	0.0	6.66
29	1.17	0.0	6.15
30	1.19	0.0	6.36
31	0.77†	0.0	
November 1*	1.05†	0.0	
2*	0.91	0.0	4.12
3*	**1.11‡**	**0.05**	**5.73**
4*	0.90	0.0	5.44
Period II. High protein diet			
5	1.12	0.0	4.35
6	1.04	0.0	5.93
7	1.15	0.0	11.08
8	1.07	0.0	10.20
9	1.08	0.0	8.05
Period III. Low protein diet			
10	1.08	0.0	8.62
11	1.13	0.0	9.03
12	1.05	0.0	7.15
13	1.12	0.0	7.50
14	1.17	0.0	7.20
15	1.18	0.0	6.93
16	**1.21§**	**0.31**	**5.64**
Period IV. Milk diet**			
17	1.12	0.10	9.36
18	1.11	0.13	15.38
Period V. Low protein diet			
19	1.02	0.00	9.38
20	1.09	0.00	6.99
21	**1.11††**	**0.16**	**7.26**

* Menstrual period.
† Incomplete collection.
‡ 0.5 gram creatine per os.
§ 2.0 grams creatine and 0.5 gram creatinine per os.
** 3 and 5½ quarts of milk on November 17 and 18, respectively.
†† 1.0 gram creatine per os in 0.5 gram doses.

of whey fed was more directly related to the degree of creatinuria than was the total protein value of the food. We have carried out one study on the effects of diets high in milk on the creatinuria of women. A weighed basal diet low in protein content was ingested during a fore period followed by a period in which casein in the form of "cottage cheese"[1] was added to the basal diet. Protein-free milk was then ingested in amounts equivalent to six quarts of milk, and finally fresh milk and milk powder equivalent to the same quantity of milk (table 6). Although in the high milk period the nitrogen excretion was four

TABLE 5

DATE	SUBJECT R., WEIGHT 51 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. High protein diet			
1919	*grams*	*grams*	*grams*
November 7	0.88	0.0	12.21
8	0.91	0.10	13.80
9	0.82	0.07	8.07
10	0.90	0.0	11.52
11*	0.88	0.0	13.28
12*	0.87	0.03	13.86
13*	0.84	0.0	11.36
14*	0.88	0.0	11.32
15	0.92	0.0	9.65
Period II. Milk diet†			
16	0.84‡	0.20	9.30
17	0.89	0.03	12.71
18	0.90	0.0	16.67

* Menstrual period.

† On November 16, 17 and 18, 2, 3 and 5 quarts of milk, respectively.

‡ Diet not strictly meat-free.

times greater than that of the basal diet period, creatine appeared only twice and in small amounts, and disappeared as the high protein diet was continued. Protein-free milk also failed to induce creatinuria. The results are in accord with those of our previous experiments.

Fate of creatine administered per os. The results of enteral administration of creatine to men are somewhat conflicting. According to

[1] The "cottage cheese" was prepared in the laboratory by the precipitation of the casein from fresh skimmed milk by weak acid, and careful washing of the precipitated casein.

TABLE 6

DATE	SUBJECT S., WEIGHT 58.6 KGM.			
	Creatinine	Creatine as creatinine	Total N	Diet
			Period I	
1920	*grams*	*grams*	*grams*	
May 1	1.02	0.0	6.27	Basal
2	1.14	0.0	8.21	
3*	0.99	0.0	5.97	
4*	0.94	0.0	5.02	
5*	0.95	0.0	5.73	
			Period II	
6*	0.88	0.0	6.85	Basal diet† and casein, 150 grams
7*	0.93	0.0	10.05	Basal diet and casein, 350 grams
8*	1.02	0.0	13.42	Basal diet and casein, 350 grams
9*	0.97	0.0	13.22	Basal diet and casein, 350 grams
10	0.97	0.0	12.72	Basal diet and casein, 350 grams
			Period III	
11	1.06	0.0	11.02	Basal diet and protein-free milk, 150 grams
12	1.14	0.0	8.32	Basal diet and protein-free milk, 180 grams
13	1.10	0.05	8.66	Basal diet and protein-free milk, 180 grams
14	1.09	0.0	8.54	Basal diet and protein-free milk, 180 grams
15	1.02	0.0	7.21	Basal diet and protein-free milk, 180 grams
			Period IV	
16	1.02	0.0	7.41	Basal diet, milk, 1 qt.; and dry milk, 100 grams
17	1.07	0.04	13.55	Basal diet, milk, 1 qt.; and dry milk, 290 grams
18	1.11	0.08	19.18	Basal diet, milk, 3 qt.; and dry milk, 267 grams
19	1.13	0.0	20.70	Basal diet, milk, 3 qt.; and dry milk, 267 grams
20	1.19	0.0	20.40	Basal diet, milk, 3 qt.; and dry milk, 267 grams
21	1.30	0.0	23.68	Basal diet, milk, 4 qt.; and dry milk, 178 grams

* Menstrual period.

† Basal diet. Breakfast: one-half orange, 75 grams bread, 1 cup black coffee. Lunch: 75 grams bread, 2 small slices fat bacon, 100 grams rice, 50 cc. cream. Dinner: 75 grams bread, 150 grams potatoes, 50 grams cheese, 1 egg, 100 grams banana, 1 cup black coffee, 50 grams butter. Total calories approximately 1750. During the periods of casein and of milk, potatoes were omitted from the diet.

Folin (12), in man on a low protein diet creatine in amounts less than 2 to 5 grams is completely retained. In a later publication however Folin and Denis (13) state that "one gram of creatine per day is about the maximum amount which a full grown man can be made to retain, even when kept on a low protein diet and on high protein diets the amounts retained are smaller than on low protein diets." Burns and Orr (14) observed creatinuria after ingestion of only 0.5 gram of creatine. Myers and Fine (15) recovered 16 to 29 per cent of administered creatine when 2 grams or less were fed, while Rose and Dimmitt (16) recovered from 5 to 10 per cent after feeding 1 to 2 grams. We have not been able to find any record of results of creatine administration to women. It is frequently implied (4), (17) that the organism of the female has less power to utilize or convert creatine when ingested than the male, but direct experimental evidence seems to be lacking.

As shown in table 2 with subject S after ingestion of 0.494 gram of creatine during menstruation the excretion of the creatine administered was almost quantitative. An equal amount of creatine fed during the intermenstrual period and in the succeeding menstrual period did not cause creatinuria nor increase the elimination of creatinine. With the same subject (table 3) ingestion of 1 gram of creatine either in one dose or divided into hourly doses of 0.1 gram to facilitate absorption resulted in the elimination of about 3 per cent in the urine. With subject A (table 4) after the administration of creatine in amounts varying from 0.5 to 2.0 grams, creatine was excreted in amounts corresponding to about 10 to 16 per cent of that ingested.

In general the results of these experiments are comparable with the results of similar experiments upon men. With the one exception mentioned above, creatine administered per os to women in amounts up to 2 grams resulted in slight creatinuria only. There seems therefore to be no marked difference in the assimilation and retention of ingested creatine by healthy men and women.

In seeking a possible cause for the excretion of creatine by women, the difference between the results of table 1 and of tables 3 and 4 is suggestive. As previously stated, during the earlier experiments (table 1) both subjects were in poor physical condition, resulting from an unusually hard year of university work. The week of May 10–17 in particular was one of physical and nervous strain, and in both subjects, especially subject A, creatinuria was observed. In contrast with the frequent occurrence of creatine in these experiments, is the infrequent elimination of creatine in the later experiments (tables 2 to 4)

which were carried out during the summer and at the beginning of the college year, when the subjects were in better physical condition. It seems possible also that the creatinuria observed at the beginning of the high protein diet (table 1, subject S, table 6) might have been related to the difficulty of ingestion of such a diet. This is borne out by the gradual disappearance of the creatinuria as the subject became accustomed to the diet. It would be of interest to compare the creatine excretion of healthy women who lead an outdoor life with that of women who work indoors under high nervous tension.

SUMMARY

1. There appears to be no direct relation between the phases of the menstrual cycle and the appearance of creatine in the urine of the adult woman.

2. Protein per se was not the causal factor in the production of creatinuria in normal woman. No evidence was obtained that there was any greater tendency toward the production of creatinuria by high protein diets during the menstrual period than during the intermenstrual period.

3. The results of the administration of creatine per os do not afford any evidence in support of the theory that the power of the adult female organism to destroy or convert ingested creatine is less than that of the male.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

VIII. On the Presence of Vasomotor Fibers in the Vagus Nerve to the Pulmonary Vessels of the Amphibian and the Reptilian Lung[1]

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Received for publication January 22, 1921

Numerous attempts have been made in the past to determine whether or not the pulmonary arteries are under vasomotor control. Most of the experimental work has been done on mammals. Because of technical difficulties the results obtained on stimulation of various nerves never gave clear and convincing evidence of the vasomotor control, for the changes in rate of flow through the pulmonary vessels or rise in pressure of the pulmonary artery as a result of stimulation of various nerves could be explained on the rate or strength of heart beat or on changes in the general arterial or venous pressure as a result of the stimulation. So uncertain were most of the results that many of the writers of textbooks do not even treat of the matter as a controversial question but incline to the belief that the pulmonary blood vessels are not under vasomotor control. Others, whom we will mention presently, felt reasonably certain that vasomotor nerves to the pulmonary vessels exist, if it will be conceded that a change in the rate of the blood flow through the isolated and perfused lung as the result of the injection of adrenalin furnishes positive proof that vasomotor nerves supply the pulmonary vessels.

LITERATURE

Our first observations on vasomotor nerves to the pulmonary vessels were made quite accidently during the experimental study of a prob-

[1] A demonstration of the presence of vasomotor nerves in the vagosympathetic to the pulmonary vessels of the frog lung (rana pipiens) was given to the members of the American Physiological Society at the Hull Physiological Laboratory, the University of Chicago, December 29, 1920.

lem of an entirely different nature. Since the results were so clear-cut and decisive we concluded that we could present data on pulmonary vasomotor nerves which were, because of the fortunate experimental conditions employed by us, quite striking and original. After we had made an extended search of the literature it became evident that many of the previous investigators and writers on this subject failed to present all of the evidence to their readers. Certainly, some of the most convincing experimental work on the subject had been overlooked by some and discredited by others, without going to the trouble of repeating it. We therefore felt that a brief chronological survey of the previous work was desirable before the presentation of our own results.

It seems that even before Claude Bernard and Brown-Séquard had demonstrated by their classical experiments the presence of vasoconstrictor nerves in the cervical sympathetic (1851) Moritz Schiff (22) noted, in 1847, a congestion of the lungs following double vagotomy which was quite different from the congestion and consolidation of the lungs of pneumonic animals. On the basis of our present knowledge his observations might be interpreted as a congestion due to the release of the pulmonary vessels from the tonic influence of vasoconstrictor nerves carried by the vagus. At any rate, the observation is interesting since it was made on the very vessels which even after the discovery of vasomotor nerves have been supposed, on the basis of experimental studies, to possess few if any vasomotor nerves of physiological consequence. In two long papers (22), (23) Schiff, on the basis of numerous and ingenious experiments coupled with accurate observations, ably defended his position against Traube in maintaining that double vagotomy effects a hyperemia of the lung which is quite different from the aspiration pneumonia which may secondarily be engrafted on the hyperemic condition, if operative procedures are not instituted to prevent it. Traube, among others, never recognized the "neuroparalytic congestion" of the pulmonary blood vessels described by Schiff following double vagotomy. On the discovery of vasomotor nerves some three years later Schiff interpreted his "neuroparalytic congestion" of the lungs in terms of a dilatation of the pulmonary vessels because of section of tonic vasoconstrictor nerves contained in the vagus of the pulmonary vessels. Forty-four years after the appearance of his first paper (24) (1894) on neuroparalytic pulmonary congestion he devised experiments to demonstrate by direct stimulation vasoconstrictor control of the pulmonary vessels through the vagus nerve. His failure to satisfactorily prove their existence in the mammal can probably be explained by a

misguiding procedure in the preparation of his animals which will be discussed more fully presently.[2]

No further work on vasomotor nerves to the pulmonary vessels is reported until Brown-Séquard (4) reopened the investigation in 1871–1873. As a result of his work physiologists focussed their attention on the thoracic sympathetic nerves as the vasomotor nerves to the pulmonary vessels; for Brown-Séquard failed to obtain any vasomotor effects on stimulation of the vagus nerve after atropinization of the animal. In 1874, Genzemer (29) confirmed the findings of Schiff and extended the work chiefly in these two directions: *a*, he maintained that the congestion of the lungs after double vagotomy appears within several hours after the operation; and *b*, stated that on exposing the parietal pleura by removal of the intercostal muscles it was possible to see with great plainness a marked reddening of the lung immediately following section of both vagi nerves (rabbits). The author concludes that tonic vasoconstrictor nerves for the pulmonary nerves are present in the vagi and that an aspiration pneumonia is a condition which often supervenes as a *secondary* phenomenon following double vagotomy. A few years later Lichtheim (18) likewise eliminated the vagus nerve as a possible vasomotor nerve to the pulmonary vessels when he failed to observe any change in the pressure of the pulmonary artery following stimulation in animals *atropinized* in order to eliminate the usual cardio-inhibitory effects incident to such stimulation. Since he obtained an increased pressure in the pulmonary artery following direct stimulation of medulla oblongata, faradization of the cervical cord, asphyxia and strychnine he concluded that the vasomotor nerves run down the cord and reach the lungs by way of the thoracic sympathetic chain rather than by way of the vago-sympathetic, a conclusion, as we have seen, previously arrived at by Brown-Séquard. In support of this position François-Franck (10) showed conclusively that stimulation of the thoracic sympathetic nerves to the lungs is followed by a *rise* in the pressure of the pulmonary artery synchronous with a *fall* in the pressure in the left auricle. He likewise concluded that the vagus contained no vasomotor fibers for the pulmonary vessels; for stimulation of the vagus nerves in animals *atropinized* to eliminate the disturbing cardiac effects had not the slightest effect on the pressure in the pulmonary artery or left auricle.

[2] Schiff finally came to the conclusion, in 1894, that the vagus carried vasoconstrictor fibers to the bronchial arteries and that the branches of the pulmonary artery received vasoconstrictor branches from the thoracic sympathetic nerves.

No one remembered or considered seriously the neuroparalytic congestion or hyperemia of the lung following double vagotomy (chiefly in rabbits) when Brown-Séquard and those who followed him demonstrated that stimulation of the vagus in animals prepared by injection of atropine to eliminate cardio-inhibitory effects was without the slightest effect on the pulmonary vessels. Nor did anyone take particular note of or seriously consider (except Schiff in 1894) two short articles by Couvreur (8), (9) which appeared in 1889. In these articles Couvreur stated that if the peripheral vagus trunk of a frog is stimulated after sectioning the cardiac fibers, one can under the microscope see a complete cessation of the blood flow through the lung.[3] He attributed these effects to the sympathetic fibers contained in the peripheral vagus trunk on the basis of the previous work of Brown-Séquard and François-Franck on the atropinized mammal; and hazarded the guess that the hemorrhages into the lung following double vagotomy might be explained on the basis of vasoconstrictor nerves carried in the vagus of some mammals. He had seen such effects one-half hour after double vagotomy in guinea pigs. It seems that he was not familiar with the similar views of Schiff outlined above and published 32 years previously.

Last year Ozorio de Almeida (30) reported a fatal congestion and edema of the lungs of double vagotomized guinea pigs following the stimulation of the peripheral ends of the vagus nerves. Double vagotomy alone without stimulation of the nerves did not effect an edema.

In the year 1890 Arthaud and Butte (1) stated that the vasoconstrictor fibers in the trunk of the vagosympathetic nerves of the frog described by Couvreur were in tonic activity; for these two investigators found that section of the vago-sympathetic nerve on one side led to a marked vasodilatation of the vessels of the corresponding lung.

Among others, Cavazzani (7) was not impressed by the statements of Couvreur and Arthaud and Butte; for the following year he refused to accept the assertion or the experimental evidence on the basis of the work reported by the physiologists mentioned above, that even light excitation of the vagus in frogs gave constriction sufficient to completely arrest the circulation through the lung. Cavazzani (1891) was apparently the first who investigated the vasomotor innervation of the

[3] When making the original observations on the frog and the turtle we were not acquainted with the work of Couvreur, Arthaud and Butte, and Krogh. As a matter of fact all of our experimental work was completed before we instituted a search of the literature which is presented in this section.

lungs by the perfusion method. Perfusing the lungs under constant pressure and then stimulating various nerves, he found that the sympathetics contained dilator and that the vagus contained constrictor nerves to the pulmonary vessels. Stimulation of the vago-sympathetic in the dog was usually followed by dilatation, however. Two years later Henriques (15) reported finding a rise in the pressure of the pulmonary artery accompanied by a fall in the pressure of the left auricle following stimulation of the vagus of a curarized cat. Since similar observations in dogs and rabbits effected a drop in arterial pressure of the pulmonary artery with rise in the left intra-auricular pressure with no change in the rate of the heart he concluded that the vagus contains chiefly dilator fibers for the pulmonary vessels of the dog and rabbit and constrictor fibers to the pulmonary vessels of the cat. This conclusion for the cat was drawn from a single experiment and the author himself questions its validity. Parenthetically, we might call the reader's attention to the fact that Henriques did not atropinize his animals prior to vagus stimulation. His work was reviewed the following year by Bradford and Dean (2) who measured simultaneously the pressure in the pulmonary and carotid arteries in morphinized, curarized, and doubly vagotomized dogs under chloroform anesthesia whilst stimulating various anterior roots of the dorsal cord within the spinal column. They obtained evidence of the presence of vasomotor (specifically vasoconstrictor fibers) for the pulmonary vessels in the 3rd, 4th and 5th dorsal nerves. They also attempted to elicit reflex constriction of the pulmonary vessels by stimulation of the intercostal, sciatic, etc., nerves. They concluded that the vasomotor system of the pulmonary vessels is but poorly developed. A search for the presence of vasomotor fibers in the vagus was unsuccessful. Following the lead set by previous investigators these authors also injected *atropine* to eliminate the cardiac effects of the vagus on the heart. No mention is made of the work of Couvreur; but the work of Henriques is mentioned as a unique example of the only author who ascribed vasomotor action to the vagus for the pulmonary vessels. During the following two years, François-Franck (11), (12), (13), (14) extended and strengthened the position held by him since the appearance of his first work in 1881. He measured simultaneously the pressure in a branch of pulmonary artery, the aorta, the left auricle and took plethysmographic records of the lung while stimulating the thoracic sympathetic trunk at various points or first thoracic nerve. He confirmed the work of Bradford and Dean in every particular. Among other things he showed that vasoconstriction

of the lung following stimulation of the sympathetic nerves was accompanied by an increase in the lung volume of the affected side together with a decrease in the left intra-auricular pressure. The increase in volume of the lungs under these conditions he attributed to the increased efficiency of the ventricular systole. By his method he demonstrated reflex vasoconstriction of the pulmonary vessels resulting from stimulation of the central ends of the crural, sciatic, and central abdominal vagus (from the latter after atropinization to prevent reflex cardiac inhibition). In fact, every afferent nerve (cutaneous or visceral) which he tried gave him the phenomenon. He considered the vasoconstriction in the pulmonary vessels as possible mechanism useful under certain conditions in preventing the arterial pressure from becoming too high by limiting the amount of blood going to the left ventricle. The matter received no further attention aside from a review of the literature on the pulmonary circulation by Tigerstedt in 1903 (27). In this review the work of Couvreur is not mentioned in the text though the bibliography includes a reference to it. Considering that an account of Couvreur's work appeared in his monograph on the physiology of the circulation published 10 years earlier (26), it would appear that Tigerstedt was not impressed with its accuracy or importance.

From this time on investigations have been restricted chiefly—almost exclusively so—to perfusion experiments of the isolated lung with a drug whose action is generally considered to be on vasomotor nerve endings, namely, adrenalin. Brodie and Dixon (3) perfused the isolated lungs of cats with cats' own blood, for they found that when they diluted the blood with Ringer's solution edema of the lungs soon supervened. In half of their experiments the lung was inflated rhythmically. The lung was perfused through the pulmonary artery. Another cannula tied to the left auricle collected the blood which issued from the perfused lung, the other lung having been tied off. As a control on the viability of the preparation under the conditions of experimentation the same methods were employed on other organs whose vessels possess vasomotor nerves easily demonstrable under better physiological conditions. These investigators failed to obtain evidence of any vasomotor control over the pulmonary vessels on stimulation of the spinal cord, thoracic sympathetic chain, loop of Vieussens, inferior cervical ganglion, vagus nerve, or the base of lung itself although under identical conditions stimulation of nerves to the hind legs or intestine gave marked evidence of vasoconstriction of the vessels of these organs. Even adrenalin was without effect in the dosage used, whereas the same dosage

produced the anticipated results in the vessels of the limbs and intestines. Pilocarpine and muscarine, which are considered universally as stimulants to nerve terminals, caused contraction of the systemic vessels but relaxation of the pulmonary vessels. They concluded, therefore, that the pulmonary vessels are devoid of any significant number of vasomotor fibers. The same year Plumier (20) confirmed some of the findings of François-Franck and furthermore stated the vasoconstrictor action following stimulation of the loop of Vieussens is bilateral; for he described a rise in the pressure of the *right* pulmonary artery with a drop in the left intra-auricular pressure on stimulation of the left sympathetic nerve (dogs). In a second paper Plumier (21) described experiments of the same nature and performed under conditions similar to those of Brodie and Dixon. In his experience stimulation of the annulus of Vieussens always caused vasoconstriction of the pulmonary vessels as did injections of adrenalin. Plumier emphasized the importance of using fresh defibrinated blood without diluting it with Ringer's solution. He furthermore suggested that the solutions of adrenalin used by Brodie and Dixon (1:20,000 in cc. doses) were too weak. Plumier used from 0.2 to 0.5 mgm. adrenalin as a dose. This dose caused enormous vasoconstriction preceded by a vasodilatation. He does not call attention to this primary dilatation. Plumier concluded that the vasomotor nerve endings in the lung were less sensitive to adrenalin than those of the leg for he could never completely stop the flow through the lungs by its use as he could by perfusion of the drug through the vessels of the leg. Some years later, Langendorff (17) showed that adrenalin caused constriction in the isolated branches of the pulmonary artery of the sheep, pig, calf and cat. This constriction was more powerful (if the tracing submitted is representative of all his experiments) than the constriction obtained on direct stimulation of the arterial wall itself with a tetanizing current.

Krogh in 1907 (16) made the following observations on the vasomotor control in the lungs of the turtle. The blood was rendered non-coagulable by hirudin and a Y-cannula placed in the pulmonary vein on one side, so that the return of the pulmonary vein blood could be temporarily prevented and the blood collected in a receptable for measurement of the blood-flow rate per unit of time. Records were also taken of the general arterial pressure. With this method of investigation Krogh found *1*, that the rate of the blood flow through the lung varied independently of the variation in the general arterial pressure; *2*, that on section of the vago-sympathetic nerve in the neck there was a great in-

crease in the rate of the blood flow through the lung of the same side, and subsequent variations in the blood flow through this denervated lung ran parallel with the changes in the general arterial pressure; *3*, that on section of the vago-sympathetic nerve on one side the rate of the blood flow through the lung of the opposite side was reduced.

Krogh concluded that the vago-sympathetic nerves in the turtle contain vasomotor nerves to the lung blood vessels, and that these (the vasoconstrictors) are in tonic activity.

The only objection that may be raised against Krogh's observations and conclusion is that the state of contraction of the lungs was not controlled.

In 1909 Wiggers (28) published experiments which essentially confirm the earlier work of Plumier.

As a general recapitulation we might state that before the discovery of vasomotor nerve fibers of any kind, Schiff (1847) suggested on the basis of experimental results that the vagi nerves carried fibers to the blood vessels of the lungs and that division of these fibers effected a neuroparalytic hyperemia of the lungs. Subsequent attempts to detect vasomotor fibers for the pulmonary blood vessels in the vagi of mammals failed except in two instances: Henriques (1893) on the basis of but few experiments definitely decided on their presence as did Schiff (1894). Henriques did not use atropine to eliminate the inevitable confusing cardiac inhibition which would itself markedly diminish or totally stop the flow of blood through the pulmonary vessels; Schiff used it to some extent but warns investigators not to use the drug in large quantities on the basis of his experience with it. All other investigators before Henriques and since then who failed to detect any vasomotor fibers in the vagus for the pulmonary vessels have without exception used this drug. (Brown-Séquard, 1871; Lichtheim, 1876; François-Franck, 1880; Bradford and Dean, 1894.) On the basis of such decisive negative evidence, Couvreur's (1889) description of vasomotor fibers for the pulmonary vessels in the vagus of the frog (really vasosympathetic) was most generally overlooked by some investigators, openly discredited by one investigator (Cavazzani, 1891), and slowly relegated to oblivion by another (Tigerstedt, 1903). That these vasoconstrictor nerves in the vagus were in constant tonic activity as stated by Arthaud and Butte (1890), received even less consideration by subsequent writers and investigators. Accordingly, later physiologists disregarded the vagus and looked for vasomotor fibers in the sympathetic nerves to the lung and found them (Bradford and Dean, 1894;

François-Franck 1891 and 1895; Plumier, 1904). Brodie and Dixon alone denied their presence (1904). Bradford and Dean (1894) and François-Franck (1896) even described experiments in which reflex pulmonary vasoconstriction was effected by the stimulation of various afferent nerves (visceral and cutaneous). Krogh (1907 and 1910) presented undubitable evidence that the vago-sympathetic nerve in the turtle contains tonic vasoconstrictor action over the pulmonary vessels. No cognizance of this work appears to have been given by any writer except Stewart (25). With the discovery of the specific hemodynamic properties and mode of action of adrenalin, the problem was again investigated by perfusing the surviving mammalian lung with blood or a blood-Ringer mixture and noting the effect of adrenalin on the rate of flow through the lung. The results, on the whole, were positive (Plumier, 1904; Wiggers, 1909) in that such perfusions reduced the rate of flow through the lung.

But this method does not decide whether the vagus or the sympathetics carry the vasomotor fibers. The assumption that the drug, adrenalin, stimulates only the myoneural junctions of the sympathetics, is in the case of our experiments, quite unwarranted; for if we can show that the vagus carries vasoconstrictor nerves to the pulmonary vessels of the frog and turtle and that these are *paralyzed by atropine*, it might be possible to explain the constrictor action of adrenalin on the blood vessels of the frog's and turtle's lung by its stimulating action on the myoneural junctions of the vagus. At any rate, from our experience with atropine on the frog and turtle it would seem highly probable that previous investigators have failed to detect the vagus vasomotor fibers for the pulmonary vessels in the mammals by paralyzing the nerve with this drug before stimulating, except Cavazzani (1891) who, using the perfusion method, described vasoconstrictors in the vagus of rabbits but dilators in the vago-sympathetics of dogs.

METHODS

Experiments on frogs. In all cases the animals were decerebrated. No anesthetics were employed. Moderate doses of curare were used in some preparations. In other cases the animals were immobilized by section of the spinal cord below the medulla and pithing it posteriorly. The animals were fixed dorsal side down, and the lungs exposed by a ventral median incision extending from the pubis to the angle of the jaw. In nearly every case the lung under observation was provided

with a cannula in the tip for recording the intrapulmonic pressure and where the experiments involved section of the vago-sympathetic nerve, the glottis was closed by forceps prior to this section, as described in our first paper on lung automatism and lung reflexes in the amphibia (5).

1. Changes in the tonus of the pulmonary blood vessels on section of the cervical sympathetic and the vagus nerves. With the glottis closed and the lungs moderately distended by inflation with air, the cervical sympathetic nerve was isolated and sectioned between the brachial plexus and the root of the vagus, the vago-sympathetic nerve was sectioned in the neck, and the changes in caliber of the pulmonary arteries produced by these sections determined by direct inspection of the lung. The section of these nerves may induce change in the caliber of the pulmonary arteries indirectly through changes in the heart rate and general arterial pressure. These factors would operate equally on the intact and the denervated lung. Hence, in this series of experiments, the blood vessels of the intact lung served as a control of the cardiac and arterial blood pressure factors.

2. Changes in the tonus of the pulmonary arteries on stimulation of the peripheral end of the cervical sympathetic and the vagus nerves. The changes in the caliber of the pulmonary vessels were determined by direct inspection. The cardiac and the arterial blood pressure factors were controlled in the following ways: *a*, tying of the heart at the sinus or excision of the heart; *b*, paralysis of the cardiac vagus with moderate doses of nicotine or curare, and recording the arterial pressure from a branch of the left aorta, in case the right lung was used for observation, or vice versa. The factor of variations in the lung tonus was controlled by moderate doses of nicotine (intravenously) or dilute solutions of atropine applied to the surface of the main pulmonary arteries. It has been shown by us previously that nicotine reverses the effects of vagus stimulation on the lung motor tissues. Hence by the use of this drug it is possible to observe the influence of the vagus stimulation on the pulmonary arteries parallel with either contraction or dilatation of the lung musculature. In the frog the main branches of the pulmonary artery run on the surface of the lung. Dilute solutions of atropine applied directly to the pulmonary artery paralyze the vagus vasomotor fibers before the lung motor fibers, so that the purely mechanical effects of lung contractions on the caliber of the pulmonary arteries may be studied.

3. Perfusion of the lung. This method was used for the study of the action of epinephrin and other drugs on the pulmonary blood vessels as

well as the influence of the stimulation of the vago-sympathetic nerve on these vessels. In these experiments the lung was left in situ, the glottis being closed and a cannula being fixed in the tip of the lung for recording the intrapulmonic pressure. The right lung was used. The arterial cannula was tied in the right aorta and all branches of this aorta ligated, save the right pulmonary artery. The main pulmonary vein, even in the common frog, is large enough for insertion of a cannula, but anatomical relations and mechanical conditions render this method impossible. For that reason we inserted the cannula for the venous outflow through the auricle into the sinus venosus. The ventricle being excised and the left aorta ligated, the only fluid entering the sinus is that from a pulmonary vein from the right side. This method of perfusion of the frog's lung works very satisfactorily, and when proper mechanical adjustments are made the drop method of venous outflow becomes a very delicate indicator of the tonus of the pulmonary vessels.

The chief difficulty lies in securing a physiological substitute for the frog's blood. Pure Ringer solution will not do. When pure Ringer's solution is perfused through the frog's lung, even under very moderate pressure, the lung passes into extreme tetanus or hypertonus within a few seconds, and in a few minutes the lung becomes edematous (as noted by previous investigators) and the lung cavity fills up with Ringer's solution and mucus. The nerves both to the lung motor tissues and to the blood vessels are quickly paralyzed. We found it necessary to add $\frac{1}{4}$ to $\frac{1}{2}$ defibrinated frog's blood to the Ringer solution in order to keep the lung tissues approximately normal for 20 to 30 minutes of perfusion.

The drugs were added to the perfusion fluid close to the inflow cannula in the aorta, and without change in the perfusion pressure. The mechanical influence from the lung contractions or relaxation following the vagus stimulation were again controlled by appropriate doses of nicotine.

4. The influence of the body temperature of the frog. The work on the frog was begun in the summer of 1920 and resumed in November and December of the same year. Frogs as secured from the dealers in this commodity vary greatly in vigor, depending probably on the length of time in captivity and the care in handling. We noted that the frogs kept in running water (8°–10°C.) during the month of December exhibited as a whole much feebler vasomotor responses in the lungs than did the frogs worked on during the summer. We therefore resorted to the expedient of slowly warming the frogs to approximately room tem-

perature (20°–25°C.) before the experiments. Raising the body temperature in this manner appeared to increase the intensity and rapidity of pulmonary vasomotor action.

5. Mechanical spread of excitation current from the vagus to the pulmonary blood vessels. We noted early in the work that the weak tetanizing current applied to pulmonary artery at the base of the lung causes extreme contraction of the artery throughout its whole length. Unless special care is taken on tetanization of the peripheral vagus there may occur an escape of the current to the carotid and by muscular conduction down the pulmonary artery. This source of error was checked up by tetanization of the carotid isolated from the vagus. The stimulation of the carotid does not change the caliber of the pulmonary arteries.

Experiments on turtles. All animals were decerebrated. No anesthetics were used, but in some cases the preparations were partially or completely curarized.

1. Changes in tonus of the pulmonary blood vessels on section of the vagi nerves in the neck. These were investigated in two ways. The lungs were isolated from the dorsal side, and the bronchi or the trachea ligated after moderate inflation of the lungs with air. In such preparations the change in caliber of the pulmonary vessels in each lung following section of the respective vagus nerve was determined by direct inspection of the lung. In another series we perfused one lung in situ, with Ringer plus defibrinated blood, care being taken in fixing the cannulae in the pulmonary artery and vein to leave the pulmonary branches of the vagus intact. The rate of blood flow through this lung was then recorded by the drop method, parallel with the record of the intrapulmonic pressure so as to control the mechanical factor involved in lung contractions and relaxations. Section of the vagus in such preparations yield objective evidence of change in the caliber of the lung blood vessels.

2. Changes in the caliber of the lung arteries on stimulation of the peripheral end of the vagus nerve. This was investigated in the following way: *a*, The respective lung was isolated from the dorsal side except for the pulmonary nerves and blood vessels, the bronchus ligated; the vagi branches to the heart sectioned or paralyzed by intravenous injection of nicotine; arterial blood pressure recorded from the central end of the pulmonary artery of the opposite side. In such preparations the effects on the pulmonary blood vessels produced by tetanization of the peripheral vagus were determined by direct inspection of the lung. In most of the preparations in this series the animals were fixed ventral side down, the plastron left intact and the lungs isolated from the dor-

sal side. In a few preparations the plastron was removed and the lungs isolated from the ventral side, in order to be sure that the possible pulmonary branches of the cervical sympathetic nerve would be left intact. These preparations were used particularly to determine the influence of the sympathetic nerves on the pulmonary arteries.

b. One lung was isolated and perfused with a Ringer-turtle blood mixture in situ, and the effects of vagi stimulation on the caliber of the pulmonary vessels determined by the change in rate of the venous flow. No attention was paid to the cardiac action of the vagus stimulation, as this could not influence the perfused lung.

c. The lung was isolated, excised, and perfused with the Ringer-blood mixture under constant pressure. The bronchus was ligated and a cannula fixed in the tip of the lung for recording the intrapulmonic pressure. During the perfusion the preparation was kept moist with Ringer solution. The drop method of venous flow recorded the influence of vagus stimulation on the pulmonary vessels, and the mechanical factor of the lung contraction controlled by inflation of the lung to a pressure equal to or exceeding that induced by tetanization of the vagus.

3. The action of epinephrin and other drugs on the pulmonary vessels. Most of the experiments in this series were made by perfusing the isolated lung (excised or in situ) with a Ringer blood mixture under constant pressure, while taking parallel records of the intrapulmonic pressure (lung tonus or contractions). In a few cases the drugs were injected intravenously and the action on the pulmonary vessels determined by direct inspection of the exposed lung, parallel records being taken of the intrapulmonic pressure and the general arterial pressure.

The drugs were introduced into the perfusion fluid near the cannula fixed in the pulmonary artery.

4. The reflex control of the pulmonary vasomotor mechanism. Only a few experiments were made on this phase of the problem. In all cases the lung under observation was isolated from the dorsal side and prepared for recording of the intrapulmonic pressure and the general arterial pressure, care being taken not to injure the pulmonary branches of the vagus nerve. Various afferent nerves were then stimulated and the effects on the lung blood vessels noted by direct inspection. In other preparations the lung, prepared as above, was perfused with the Ringer-blood mixture, the reflex effects from sensory stimulation on the lung arteries recorded by the rate of the venous outflow.

For a more detailed account of our method of preparing the turtle for observing and recording the lung rhythms, the reader is referred to an earlier paper in this series (6).

RESULTS ON THE FROG

1. The effects on the tonus of the pulmonary arteries of ligation of the vagus and the cervical sympathetic nerve. When the frog is prepared and the lungs exposed for the study of the lung automatism and the lung reflexes, as described by us in an earlier report, the pulmonary arteries on the two lungs show usually an equal degree of dilatation, as nearly as this can be determined by direct inspection. Occasionally the arteries of one lung are more constricted than on the opposite lung, and in some preparations there is periodic variation in the size of the lung arteries, like Traube-Hering waves of blood pressures. These periodic variations in the caliber of the lung arteries may or may not be synchronous in the two lungs. Blood pressure records were not taken in these animals. Under these conditions the periodic variations in the size of the pulmonary arteries that were synchronous in the two lungs could have been due to changes in the strength of the heart beat and consequent changes in the arterial pressure, that could not be detected by the method of direct inspection. It was not due to changes in the rate of the heart. But this explanation fails to account for the tonus variations in the arterial vessels that were not synchronous in the two lungs. In many of the preparations the glottis was closed and graphic records taken of the lung tonus and the lung contractions. In this way we determined that the periodic changes in the caliber of the pulmonary arteries were independent of the tonus changes in the lung musculature.

In these preparations ligation of the cervical sympathetic nerve had in no instance any influence on the caliber of the pulmonary arteries either on the same or on the opposite side. But *ligation or section of the vagus nerve usually caused, on the same side, a temporary constriction of the lung arteries followed by a permanent dilatation.* The temporary vasoconstriction was noted less frequently than the permanent dilatation. It is obvious that both the temporary constriction and the permanent dilatation of the arteries had to be marked in order to be made out with a certainty by direct inspection. In some of our preparations the permanent dilatation of the lung arteries following the ligation of the vagus nerve on the same side was as marked as the hyperemia of the ear after section of the sympathetic nerve in the rabbit.

It should be noted that ligation of the vagus produces the above effects on the pulmonary vessels of the same side only. The fact that the caliber of the arteries of the intact lung is not influenced by section of the vagus on the opposite side eliminates the heart and the blood

pressure as factors in the vasodilatation of the denervated lung. But ligation of the vagus induces a permanent hypertonus of the lung on the same side. Before we may conclude on the basis of these experiments that the vagus contains vasoconstrictor fibers to the lung and that this vasoconstrictor mechanism is in tonic activity, it is necessary to show that the vasodilatation is not an effect of the lung hypertonus. It is conceivable that the lung contraction may induce dilatation of the lung arteries as a mechanical effect of capillary compression and hence increased resistance. Or, the vasodilatation may be a type of peripheral physiological coördination, the greater muscular work in the lung effecting local vasodilatation by diffusion of tissue metabolites. The complete answer to these possible explanations is secured by another line of experiments. But the following facts may be stated here. In some preparations the ligation of the vagus caused hypertonicity of the lung but no vasodilatation that could be detected by direct inspection. This shows that the pulmonary vasodilatation is probably not due to lung hypertonus, as the latter may be induced without the former.

The obvious conclusions from these experiments seem to be that the vagus (but not the sympathetic) contains pulmonary vasoconstrictor fibers, and that this mechanism is in tonic activity. The degree of this tonic activity will naturally vary with the condition of the frog, the trauma of the operation, degree of asphyxia, etc.

2. The influence on the pulmonary arteries of stimulation of the peripheral end of the vagus and the sympathetic nerves. This was determined by direct inspection, and by the rate of venous flow in the perfused lung. In the case of the first method the cardiac factor was controlled by curare or nicotine.

Typical tracings illustrating the results obtained by the first method are reproduced in figures 1 and 2. It will be noted that tetanization of the peripheral vagus (after nicotinization) induces complete constriction of the lung arteries, with no change in pulse and arterial pressure or with a synchronous acceleration of the heart and rise in blood pressure (stimulation of the accelerator nerves), and independent of the lung contraction and lung inhibition. These experiments eliminate the heart, the blood pressure and the lung tonus as factors in pulmonary vasoconstriction. We are obviously dealing with a true vasomotor nerve mechanism. In good preparations the obliteration of the pulmonary arteries on tetanization of the peripheral vagus is as complete as the blanching of the ear on stimulation of the cervical sympathetic

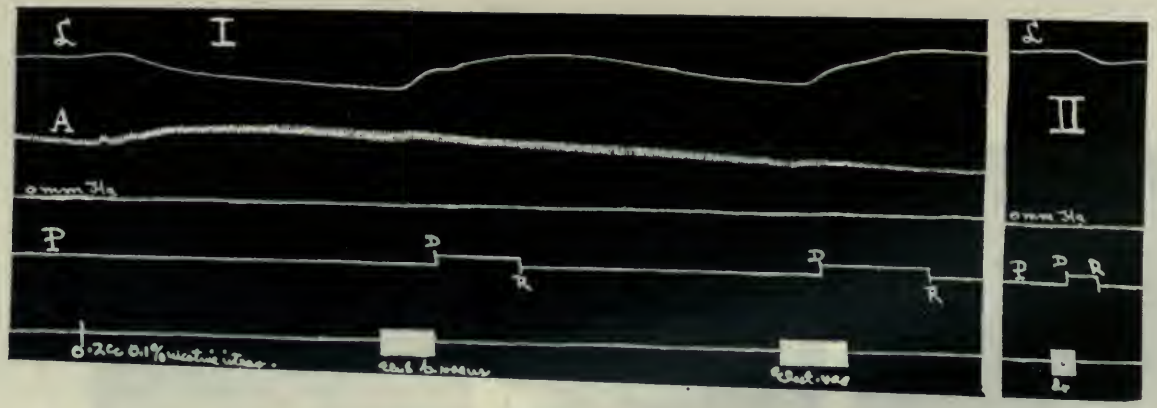

Fig. 1.[4] Record of the intrapulmonic pressure of the right lung, *L*, left aortic blood pressure, *A*, constriction of the pulmonary vessels, *P*, where at *D* the pulmonary vessels disappeared completely to return with maximum pulsation at *R*, as a result of stimulation of the peripheral end of the vagus after intravenous injection of 2 mgm. nicotine as indicated by the signal magnet tracing of the lowermost line. Frog decerebrated, spinal cord transected below the medulla and pithed. Intrapulmonic pressure from tip of right lung; glottis closed with hemostat.

Showing in I a complete *disappearance* of the pulmonary vessels as a result of stimulation of the peripheral end of the vagus and the subsequent reappearance of the pulmonary vessels at a time when the aortic pressure and rate of heart beat were quite steady and when the intrapulmonic pressure was considerably higher than at any period prior to the vagus stimulation.

Showing in II complete blanching of the blood vessels of the lung of the same frog on vagus stimulation with arterial pressure at zero because of excision of the heart at a time when the lung showed inhibition. These tracings are offered to illustrate the complete independence of the cardiac, pulmonary and vasomotor effects on stimulation of the peripheral end of the vagus.

[4] The figures in this article have been reduced from one-half to one-fourth their original size.

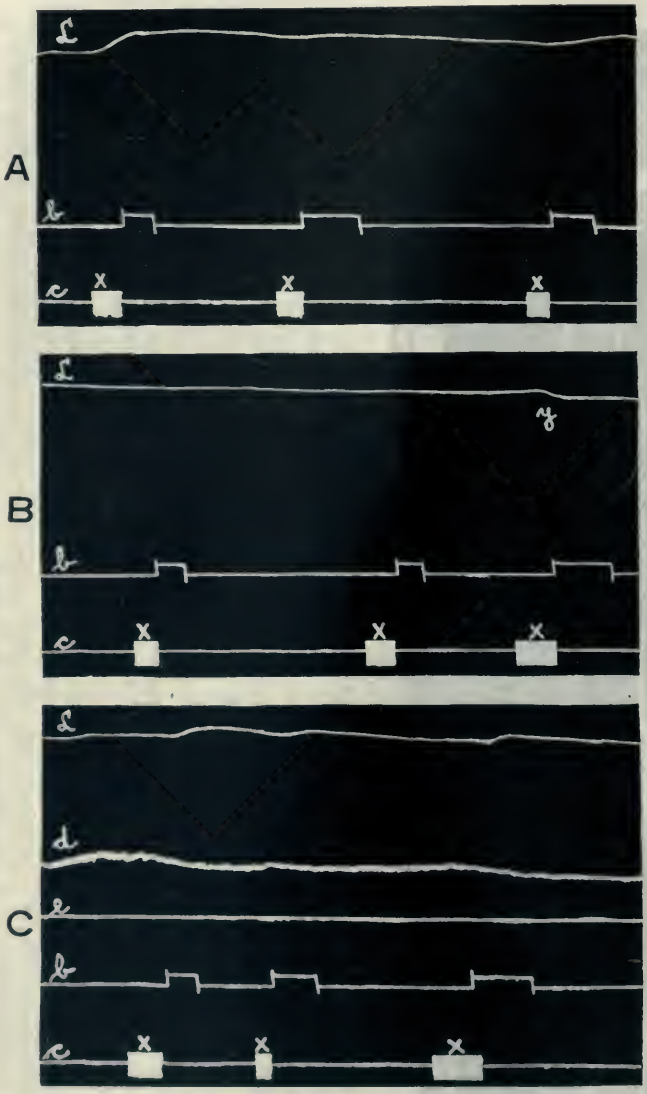

Fig. 2. Simultaneous records of the intrapulmonic pressure, constriction of the pulmonary arteries and general arterial blood pressure of a frog on tetanization of the peripheral end of the vago sympathetic after the intravenous injection of 2 mgm. nicotine. Frog decerebrated, spinal cord transected below the medulla and pithed. Cannula in tip of right lung, glottis closed with mosquito forceps. Intravenous injection through the abdominal vein. Blood pressure from left

nerve, in the mammal (rabbit, cat). By our method the vagus-lung vasomotor mechanism is as readily demonstrated as the vasomotor nerves to the ear.

All of our experiments attempting to influence the tonus of the pulmonary blood vessels by stimulation of the peripheral end of the cervical sympathetic nerve yielded negative results. Since stimulation of the cervical sympathetic resulted in cardiac acceleration without a vasoconstriction of the pulmonary vessels, it is reasonable to assume that the vasomotor nerves were not injured in the isolation of the sympathetic. The pulmonary vasomotor fibers in the vago-sympathetic nerve belong therefore essentially to the vagus system. If the cervical sympathetic nerves contain any vasomotor fibers to the pulmonary vessels our method of direct inspection fails to reveal them.

Tracings showing the effect of vagus stimulation on the flow of blood through the perfused lung are reproduced in figures 3 to 5. It will be noted that tetanization of the peripheral vagus retards or stops the blood flow through the lung, irrespective of whether the stimulation induces an increase or a decrease in the lung tonus. The pulmonary vasomotor mechanism thus works independently of the lung motor mechanism. In fresh preparations tetanization of the vagus causes sufficient vasoconstriction to stop completely the blood flow through the lung. But this vasomotor mechanism fails quickly in the perfused preparations.

aortic arch. In all tracings L = record of intrapulmonic pressure; b = signal magnet tracing in which the upstroke indicates the *complete* constriction of the pulmonary arteries as determined by direct observation and the downstroke their complete reappearance; c = signal magnet tracing indicating at x stimulation of the peripheral end of the vagus; d of tracing C is a record of the aortic pressure, e being the 0 mm. Hg. pressure.

In A the constriction of the pulmonary vessels on stimulation of the vagus at x occurs during or is associated with a contraction of the pulmonary musculature.

In B the complete vasoconstriction on vagus stimulation occurs without any significant change in the intrapulmonic pressure or even with a slight inhibition of the lung, y, together with a cardiac acceleration.

In C stimulation of the peripheral vagus at x caused a complete constriction of the pulmonary vessels accompanied in several instances by slight rise in the aortic pressure (cardiac acceleration) and a slight rise in the intrapulmonic pressure, which in every instance comes on after the vasoconstriction is complete and outlasts the vasoconstriction.

Showing the independence of the cardiac, the vasoconstrictor and lung motor action of the vagus.

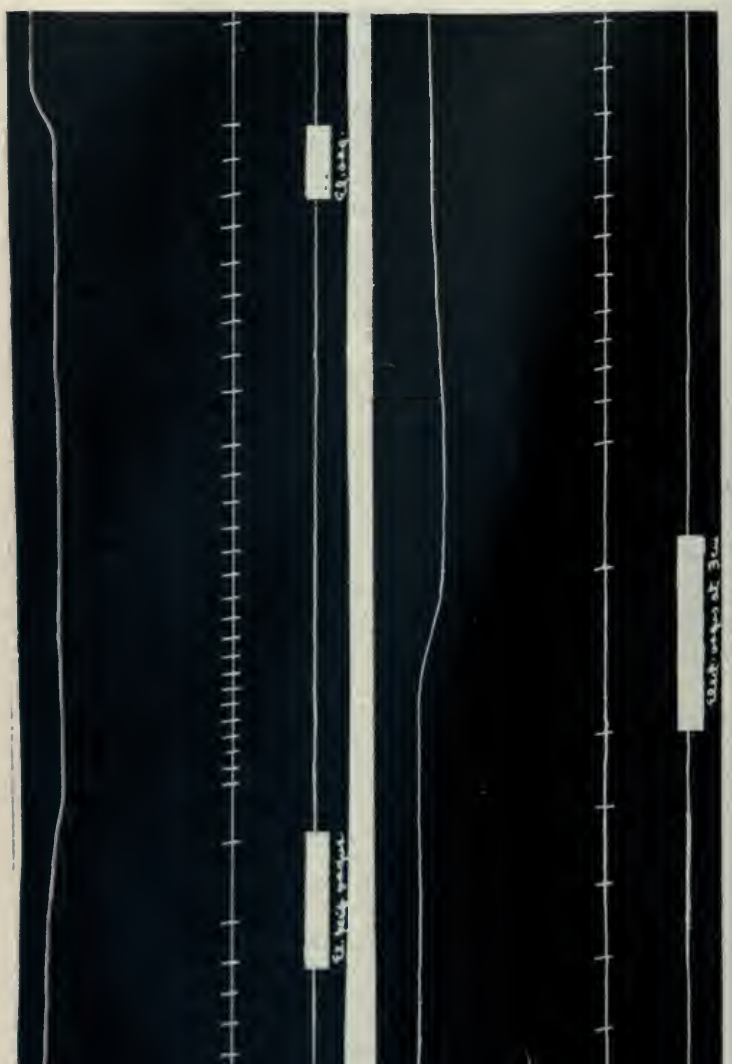

Fig. 3. Record of the intrapulmonic pressure of the right lung of frog, *L*, with signal record recording drops of blood-Ringer mixture issuing from the same lung, *P*, with lowermost signal magnet tracing indicating electrical stimulation of the peripheral end of the right vagus. Frog decerebrated, spinal cord transected below the medulla and pithed. Perfusion of the right lung through left aortic arch, all vessels other than right pulmonary artery (including the bulbus aortae) having been tied off. Outflow from the lung measured in drops of fluid issuing from a cannula tied in the auricle.

Showing a diminution in the outflow from the perfused lung on stimulation of the vagus followed by a temporary acceleration associated either with a contraction or inhibition of the lung.

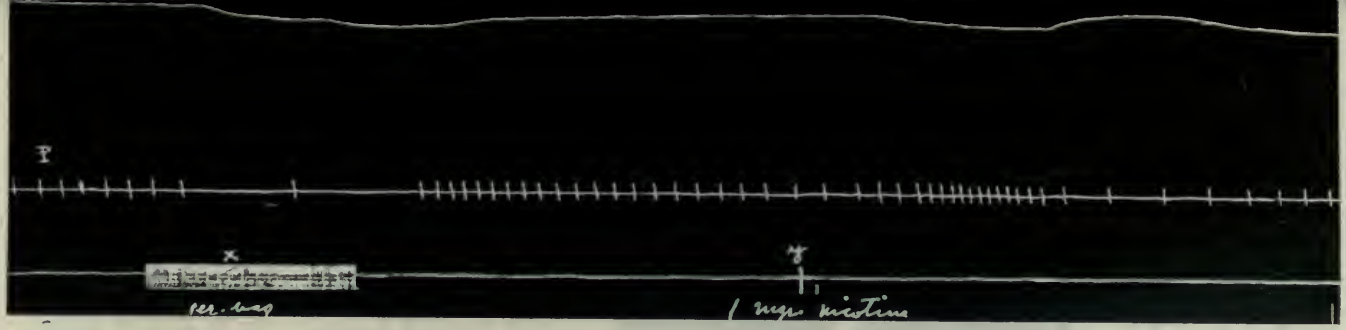

Fig. 4. Record of the intrapulmonic pressure, L, of the right lung of frog with record by means of signal magnet, P, of drops of blood-Ringer mixture issuing from the same lung with lowermost line indicating electrical stimulation of peripheral end of the right vagus at x and the perfusion at y of the same lung with 1 mgm. of nicotine.

Animal prepared as described in the text and in legend of figure 3.

Showing extreme diminution in rate of outflow from the lung on electrical stimulation of the vagus associated with an inhibition of the lung; also primary acceleration and, secondarily, a marked diminution in the rate of outflow as the result of the perfusion of the lung with nicotine.

Fig. 5. Record of the intrapulmonic pressure of the right lung of the frog, L, with signal magnet record indicating drops of blood-Ringer mixture issuing from the same lung, P, and lowermost line indicating stimulations of the peripheral end of the vagus at x and the perfusion of the lung at y with $\frac{1}{10}\frac{1}{0}$ cc. 1:1000 adrenalin chloride solution. Animal prepared as described in the text and in the legend of figure 3.

The record shows temporary diminution in rate of flow from the lung on stimulation of the vagus as well as primary increase, and then marked diminution in the rate of outflow as the result of the perfusion of the lung with adrenalin chloride in the absence of any significant changes in the intrapulmonic pressure.

In none of our preparations and with no strength or type of stimulation of the peripheral vagus did we obtain primary dilatation of the pulmonary blood vessels. This does not necessarily mean that vasodilator nerves are absent. It does mean, at least, that the constrictor mechanism is preponderant.

3. The effect of epinephrin and histamine on the pulmonary blood vessels. It would seem that the facts recorded in the foregoing sections

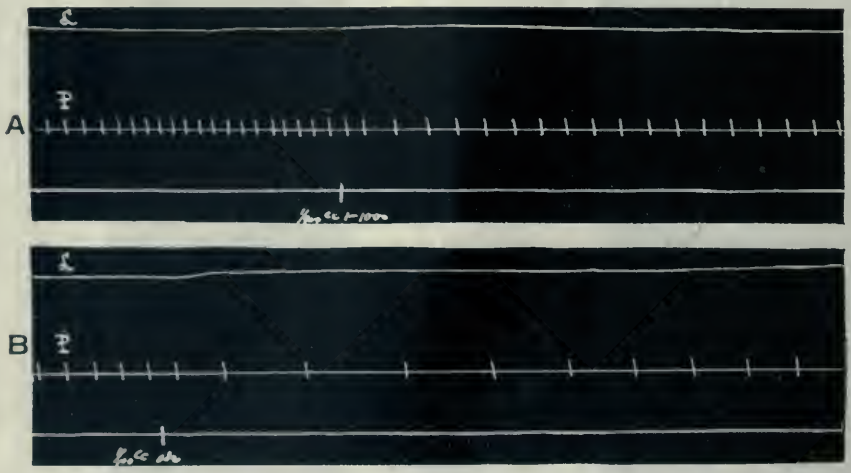

Fig. 6. Records of the intrapulmonic pressure, *L*, and venous outflow of the right lung of the frog in drops of frog blood-Ringer's mixture (mostly Ringer's), *P*, with lowermost line indicating by signal magnet the injection of the drug, adrenalin chloride. Perfusion of the right lung by way of the left aortic arch, all other blood vessels distal to the right pulmonary artery (including the bulbus aortae) having been tied off. The perfusion mixture returning from the lung was collected by a cannula tied into the auricle through the sinus. All injections made by hypodermic needle at cannula of inflow into the lung. Showing in A slight vasoconstrictor action following the perfusion of the lung with $\frac{1}{200}$ cc. of 1:1000 adrenalin solution; in B, pronounced vasoconstrictor action on doubling the dose.

are sufficient proof that the blood vessels of the frog's lung are supplied with vasomotor nerves through the vagus system. Nevertheless a number of experiments was made with the action of epinephrin on the perfused lung, partly because of the contradictory results obtained from epinephrin by previous investigators in the case of the mammalian lung, and partly because of the prevalent view that epinephrin is specific for sympathetic nerve endings, and in case of the pulmonary blood vessels

of the frog the vasomotor nerves most probably belong to the vagus system, not to the sympathetic. Will epinephrin act on the vagus nerve endings?

Typical records showing the effect of epinephrin on the tonus of the pulmonary blood vessels of the frog are reproduced in figures 5 and 6. Primary vasodilatation from small doses of epinephrin is obtained only in very fresh preparations. The prolonged vasoconstrictor action from larger doses of the drug can be demonstrated in a perfused preparation over a somewhat longer period. But the blood vessels of the perfused lung soon (10 to 20 minutes) cease to react to epinephrin in any concentration. But there is no question of the vasoconstrictor action of epinephrin in moderately large doses in the fresh preparation.

In comparing our results on the perfused lung with those of previous investigators on perfusion of the frog's hind legs, it seems that the pulmonary vessels are less sensitive to epinephrin than are the blood vessels of the extremities, a conclusion previously arrived at by Plumier (21) during similar perfusion experiments of the mammalian lung. We believe this difference is only apparent and due to the rapid deterioration of the lung tissues under perfusion, as compared to the extremities. In any event, epinephrin has the same action on the lung arteries as on the arteries of the systemic circulation, despite the fact that the pulmonary vasomotor nerves belong to the vagus system.

The action of histamine: We have reported elsewhere that intravenous injection of histamine in the frog may cause lung dilatation or lung contraction, depending on the concentration of the drug and the physiological condition of the local nervous system in the lung. If the perfused lung is in fresh condition the pulmonary blood vessels are more sensitive to histamine than the lung motor tissues, that is, concentrations of histamine that have little or no action on the lung tonus cause a marked and prolonged contraction of the lung blood vessels (fig. 7). It should also be noted that the perfused lung very soon fails to respond to histamine in any concentration.

4. The paralysis of the pulmonary vasomotor nervous mechanism by atropine. The discovery that atropine abolishes the vasomotor action of the vagus nerve on the lung arteries, made early in the work, created at first a very perplexing situation. So far as we know, atropine does not paralyze vasomotor nerves in any other organ in any species of animals investigated. We observed the fact in attempting to eliminate the cardiac factor (on vagus stimulation) by atropine, following the lead of nearly all previous workers on the mammalian lung. Prepara-

Fig. 7. Record of the intrapulmonic pressure of the right lung of a frog L, and rate of perfusion in drops, P, issuing from the same lung before and after the perfusion of the lung with 1 mgm. of histamine as indicated by signal magnet on lowermost line of the tracing.

Frog decerebrated and spinal cord transected and pithed below the medulla. Cannula in tip of right lung, glottis closed with hemostat. Perfusion through left aortic arch, all blood vessels except right pulmonary artery ligated off. Bulbus aortae ligated off. Return flow from lung through cannula tied in auricle of heart.

Showing marked vasoconstriction of the pulmonary vessels with subsequent return following perfusion of the lung with histamine.

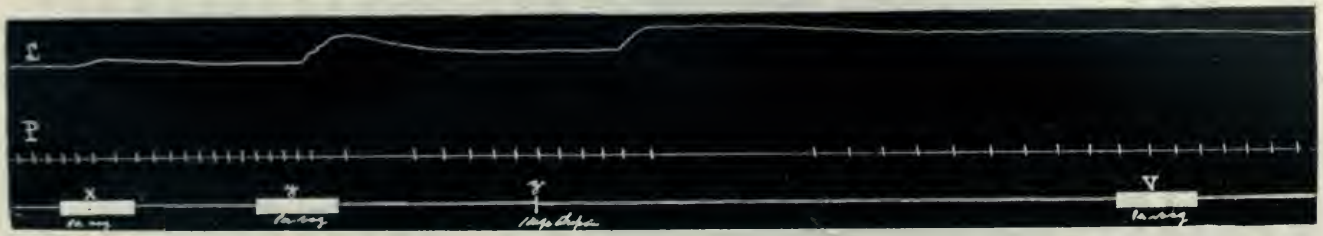

Fig. 8. Record of the intrapulmonic pressure, L, of the right lung of a frog with signal magnet record, P, of drops of blood-Ringer mixture issuing from the right lung.

Animal prepared as described in text and in legend of figure 7.

x = Stimulation of vagus with weak tetanizing after nicotinization of the lung.

y = Same, using stronger current.

z = Perfusion of the lung with 1 mgm. atropine sulphate.

V = Electrical stimulation of the peripheral end of the vagus after atropine. Showing that the vasomotor and lung motor action of the vagus is abolished by atropine.

tions that showed marked vasoconstriction in the lung on vagus stimulation failed to show this effect after sufficient atropinization to paralyze the cardiac vagus. Now, as atropine also abolishes the lung motor fibers in the vagi, the suggestion was obvious that our apparent vasomotor action in the lung is in some way due to change in the lung tonus. This unexpected action of atropine called for special scrutiny of our experiments, and devising of additional tests to place the matter beyond controversy. We believe this has been done in the following way: When atropine is injected into the circulation the lung motor and the vasomotor fibers are paralyzed practically at the same rate (fig. 8), but

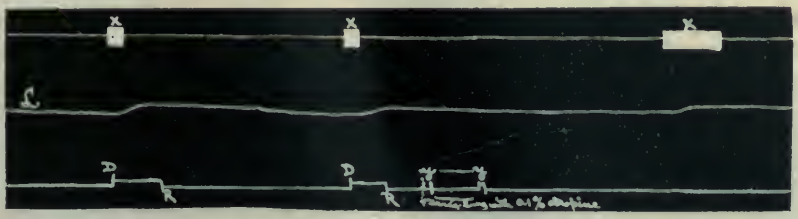

Fig. 9. Record of the intrapulmonic pressure, L, of the right lung of a frog with signal magnet record indicating at D complete disappearance and at R complete reappearance of the large superficial pulmonary vessels on electrical stimulation of the right peripheral vagus at x before and after irrigation of the lung (y–y) with several drops of 0.1 per cent solution of atropine sulphate.

Frog decerebrated, spinal cord transected below medulla and pithed. Cannula in tip of right lung. Cardio-inhibitory effects of vagus abolished with nicotine introduced into the pericardial sac. At x, electrical stimulation of the peripheral end of the vagus.

Showing paralyzing action of atropine on the vasomotor fibers before the lung motor fibers and indicating furthermore that the contraction of the lung musculature itself is not responsible for the complete vasoconstriction seen before the application of atropine.

when a few drops of dilute atropine solution are painted directly on the pulmonary arteries, the vasomotor nerves fail before the lung motor nerves. A record illustrating this selective action is reproduced in figure 9.

These results project two discordant facts into the foreground of the pharmacology and physiology of epinephrin and atropine, the latter paralyzing the pulmonary vasomotor nerves, and the former exhibiting the typical hemodynamic action on blood vessels that are innervated, not by the sympathetic but by the vagus. We are sure of the facts, no matter how divergent they are from the prevalent views of the chemotaxis of these drugs.

5. Vasomotor reflexes in the lungs. Having demonstrated the presence of vagal vasomotor nerves to the pulmonary arteries and their tonic activity, we naturally became interested in the reflex control of this mechanism. All our observations on this phase were made on preparations sufficiently curarized to eliminate the readily elicited cardio-inhibitory reflex, and direct inspection of the lung arteries had to be relied on to determine reflex effects. It needs scarcely to be pointed out that direct inspection can detect marked vasoconstriction or vasodilatation only. Slight vasomotor changes will escape detection.

In decerebrated and lightly curarized frogs marked reflex vasoconstriction can usually be produced in the lung by tetanization of the central end of the opposite vagus or the central end of the brachial nerve on either side. This probably occurs against a rise in general arterial pressure. Reflex vasodilatation in the lung could never be detected by our method of observation. Theoretically, it should occur even in the absence of vasodilator nerves, through central inhibition of the tonus of the vasoconstrictors.

We have been impressed by the difficulty in securing reflex vasomotor effects in the frog's lungs in comparison with the ease that lung motor, cardiac, and skeletal reflexes are obtained in this species from all afferent nerves. But this difference may be only apparent or due to the failure of noting slight changes in the caliber of the pulmonary arteries by direct inspection.

RESULTS ON THE TURTLE

1. Influence on the pulmonary blood vessels of section of the vagi and the sympathetic nerves. In several preparations the pulmonary arteries were found markedly constricted after isolation of the lungs by our method of preparation. This might be due to mechanical injury to the lung arteries in the process of lung isolation. We have previously called attention to the fact that in the turtle mechanical handling of even a large artery like the carotid may cause extreme tonic constriction of the artery lasting for 10 to 30 minutes. The pulmonary arteries behave in the same manner. It is therefore necessary to exercise great care in isolating the lungs so as not to subject the lung arteries to rough mechanical handling. The type of tonic arterial constriction due to direct mechanical stimulation is always local or confined to the region of the artery subject to direct handling. This fact renders it a priori improbable that a tonic vasoconstriction

extending uniformly over the entire lung is due to direct mechanical injury. In such preparations *section of the vagus nerve in the neck leads to a prompt and lasting vasodilatation in the lung of the same side.* This is not due to improvement in the heart and a higher arterial pressure. Were this the cause, the vasodilatation should occur in both lungs. The fact is that it is always confined to the lung on the side of the vagus section. It also occurs in preparations in which the vagus section does not augment the heart beat or raise the arterial pressure.

Section of the cervical sympathetic had in no case any effect on the caliber of the pulmonary vessels. Moreover, section of the vagus close to its exit from the skull has the same effect as section of the nerve further peripherally after the cervical sympathetic nerve has joined the vagus trunk. These experiments with direct observation have

Fig. 10. Perfusion of left lung (in situ) of a decerebrated and curarized turtle, fixed dorsal side down and plastron removed. Left vagus intact; perfusion fluid, Ringer's defibrinated turtle blood. *L*, record of intrapulmonic pressure; *V*, rate of venous outflow (drops). *a*, section of left vagus in the neck. Showing vasodilatation in the lungs following section of the vagus.

been corroborated on lungs perfused in situ, but with the vagus nerve intact. A typical record showing about 75 per cent increase in the rate of blood flow through the lung following section of the vagus nerve is reproduced in figure 10.

In most of our experiments records were taken of the intrapulmonic pressure. The section of the vagus nerve produces no changes in the lung tonus or lung contraction that can account for the permanent dilatation of the pulmonary vessels. We must therefore conclude that the pulmonary arteries in the turtle are provided with vasomotor nerves through the vagi, and that this vasomotor mechanism is, at least in some animals, in tonic activity. In our most favorable preparations the hyperemia of the lung following section of the vagus nerve is as marked as the hyperemia of the ear following section of the cervical sympathetic nerve.

But why should we not be able to demonstrate this pulmonary-vasomotor tonus in all of our preparations? In many animals the section of the vagus nerve did not appreciably alter the caliber of the pulmonary arteries. This was particularly true in animals in poor condition from long captivity or other causes. We were probably also dealing with the factor of traumatic and nervous shock, unavoidable from the method of preparing the animals. The temperature of the animal may be a factor as it is in the frog. We have only worked on turtles taken from cold water. In no instance did we warm up the turtles as we did the frogs before preparing them for experimentation.

2. Pulmonary vasomotor rhythm. In some of our preparations periodic alterations in the caliber of the pulmonary arteries were noted similar to those described for the frog. In the turtle this was seen both in the intact and the denervated lung. The mechanism of this rhythm is therefore partly peripheral. The periodic changes in the caliber of the arteries is so marked that there is no mistaking it even with direct inspection. This peripheral vasomotor rhythm is independent of the tonus of the lung motor tissues (fig. 11). This vasomotor rhythm was seen more frequently in the perfused lung than in lungs with the normal circulation intact. Similar periodic changes in the caliber of the pulmonary vessels of the mammalian lung were noted by Cavazanni (7).

3. The influence on the pulmonary blood vessels of stimulation of the vagus and the sympathetic nerves. This phase of the question was investigated on isolated lung perfused in situ or after excision from the body, as well as by direct inspection of the lung with the circulation intact. In every case controls were run on the lung contraction induced by the vagus stimulation. Typical records illustrating the results obtained are reproduced in figures 12 to 15. Weak tetanization of the peripheral vagus in a perfused lung may cause vasodilatation (fig. 12). But in most of the preparations all strengths of vagus tetanization that had any effect on the pulmonary circulation at all, caused vasoconstriction (figs. 13 and 14). The strength of the tetanizing current required to influence the pulmonary vessels when applied to the peripheral vagus causes at the same time contraction of the lung musculature. The lung, excised or in situ, perfused with a mixture of Ringer plus defibrinated turtle blood deteriorates gradually, though not as rapidly as the perfused lung of the frog. In a perfused turtle lung gradually deteriorating the pulmonary vasomotor mechanism fails long before the failure of the lung motor mechanism. In fact some preparations showing good vasoconstriction of the lungs on vagus stimulation when the

Fig. 11. Perfusion of excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of intrapulmonic pressure (lung tonus). *V*, rate of venous outflow in drops. Showing peripheral spontaneous variations in tonus of the pulmonary blood vessels. These tonus variations were evident on direct inspection of the lung.

Fig. 12. Record of lung tonus and lung perfusion in the turtle. Animal decerebrated, fixed dorsal side down, plastron removed, and viscera lifted so as not to press on lung. Perfusion of Ringer + defibrinated turtle blood through left pulmonary artery. *V*, rate of pulmonary venous outflow (drops). Signal, tetanization of peripheral end of left vagus, showing vasodilatation parallel with the lung tetanus.

Fig. 13. Excised lung of turtle, perfused with Ringer's defibrinated turtle blood. Bronchus, ligated, cannula in tip of lung. *L*, record of lung tonus. *V*, rate of venous outflow in drops. Signal, tetanization of lung vagus. *a*, with secondary coil at 15 cm.; *b* and *c*, at 10 cm. Showing lower excitability and more rapid fatigue of the pulmonary vasomotor mechanism than of the motor mechanism of the lung itself.

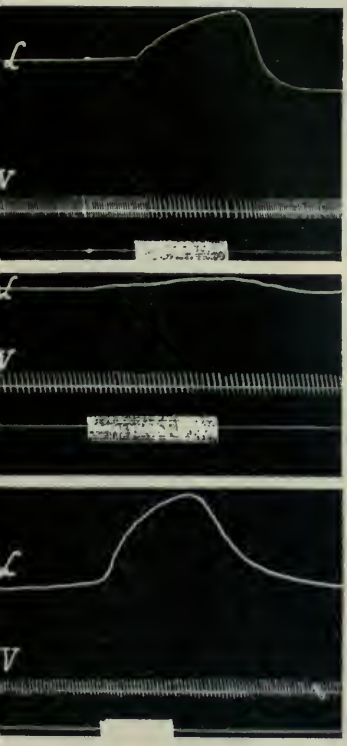

Fig. 14

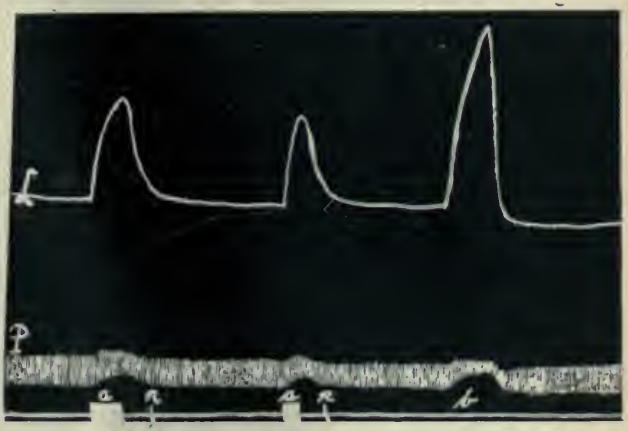

Fig. 15

Fig. 14. Excised lungs of the turtle, perfused with Ringer's solution + defibrinated turtle blood. Bronchus ligated, cannula in tip of lung. *L*, record of lung tonus. *V*, rate of venous outflow in drops. Signal, tetanization of the lung vagus. A and B, records from left lung in fresh condition, ½ mgm. atropin sulphate injected into perfusion solution near the pulmonary artery between records A and B. C, record from right lung of the same turtle 26 hours later. Showing pulmonary vasoconstrictor action of the vagus *A*, paralysis of this action by atropin *B*, and more rapid failure of the vasomotor mechanism than of the lung motor mechanism as the lung is placed in non-physiological conditions, *C*.

Fig. 15. Records of the intrapulmonic pressure, *L*, and arterial blood pressure, *P*, (right pulmonary artery) in the turtle. Animal decerebrated and curarized. Left lung isolated from the dorsal side, the bronchus ligated, and the cannula for recording the intrapulmonic pressure fixed in the tip of the lung. Left vagus sectioned in the neck, the cardiac and gastric branches of the vagus sectioned, leaving only the pulmonary branches intact.

a, Stimulation of the peripheral end of the left vagus with a weak tetanizing current. The end of tetanization indicates the complete disappearance of the pulse in the arteries of the left lung; *r*, reappearance of the pulse in the left lung; *b*, raising the intrapulmonic pressure (left lung) by air inflation above that due to the lung contraction following tetanization of the vagus. This artificial raising of the intrapulmonic pressure did not obliterate the pulse in the lung arteries.

circulation was left intact, failed to respond with vasomotor action when artificial perfusion had been established. Even in our best preparations the vagus acts on the pulmonary arteries only during the first 10 to 15 minutes of the perfusion, while the vagus lung motor mechanism may persist for hours in the perfused lung. When the peripheral vagus (the cardiac fibers being sectioned) is tetanized with the lung in situ and the circulation intact, there is produced so marked a vasoconstriction in the lung that the pulse disappears even in the large branches of the pulmonary arteries (fig. 15), but the constriction is rarely great enough to completely obliterate the lumen of these arteries, as is the case in the frog's lung.

We have been unable to influence the lung blood vessels by the stimulation of the cervical sympathetic nerve. We have stimulated this nerve intact and with the vagus intact; also the peripheral end with the vagus intact, and the central end both with sectioned and intact vagus, all with equally negative results no matter whether the lungs were isolated from the ventral or the dorsal side. It would therefore seem that in the turtle, as in the frog, the pulmonary vasomotor nerves belong to the vagus system.

We have stated, and accompanying tracings show the fact, that in all cases where tetanization of the peripheral vagus causes pulmonary vasomotor action there is also contraction of the lung. Is there not a possibility that the vasomotor changes are mechanical effects due to the contraction of the lung musculature? This question can be answered in the negative, for the following reasons:

a. In preparations in which the vasomotor nerves have failed owing to poor physiological condition the vagus stimulation causes lung contraction, but no change in the caliber of the blood vessels. Hence lung contractions do not necessarily produce demonstable changes in the caliber of the blood vessels or the rate of the blood flow (figs. 13, 14).

b. Increasing the intrapulmonic pressure artificially (by inflation) even above that caused by the active lung contraction does not obliterate the lung arterial pulse, at least not to the same extent that it is done by the vagus stimulation (fig. 15). Of course, raising the intrapulmonic pressure by inflation produces different mechanical conditions on the lung blood vessels than those produced by the lung contractions. And it is possible to raise the intrapulmonic pressure by inflation to such a degree that the arterial pulse of the intact lung is weakened, and the flow of blood through the perfused lung retarded.

c. Certain drugs (epinephrin, histamine, pituitrin) act both on the lung motor and the lung vasomotor mechanism, but the actions on these two mechanisms do not run parallel. For example, epinephrin, causes vasodilatation or constriction parallel with or without relaxation of the lung musculature. Histamine causes vasoconstriction and lung contraction, but the vasoconstriction persists longer than the lung contraction.

d. On theoretical ground it seems possible that the active lung contraction by pressure on lung capillaries and veins may temporarily augment the venous outflow, and thus give the effects of vasodilatation. We have tested this possibility by occluding the lung artery prior to the tetanization of the vagus in the perfused lung. Under these conditions the lung contraction caused by the tetanization of the vagus nerve does not start up a temporary venous outflow from the lung. It seems therefore clear that tracings like that reproduced in figure 12 cannot be due to this mechanical factor.

It is true that the relation of the main lung arteries to the lung musculature is not as simple in the turtle as in the frog. In the frog the main branches of the lung arteries run on the surface of the lung; in the turtle these arteries, at least in part of their course, pass through the body of the lung, and may therefore be more directly influenced by the tonus of the lung musculature. But the facts enumerated (*a*, *b*, *c*, *d*) show conclusively that the vasomotor changes in the lung following tetanization of the peripheral vagus are direct vasomotor effects and not mechanical products of the changes in tonus of the lung musculature.

4. Action of epinephrin, histamine and pituitrin on the pulmonary blood vessels. The action of these drugs was studied both by introduction into the general circulation and by addition to the perfusion mixture of the isolated lung. The latter method gives the most satisfactory data as it eliminates the change in general arterial pressure and the uncertainties of direct inspection.

If the perfused lung is in good condition the weakest concentration of epinephrin that affects the lung blood vessels at all causes vasodilatation (figs. 16, 17). If the lung blood vessels are in marked tonus the vasodilatation caused by the minimal quantities of epinephrin may increase the rate of the blood flow through the lung several hundred per cent. Larger quantities of the drug have the opposite effect or a vasoconstrictor action, which in fresh preparations may last 10 to 15 minutes (fig. 18). The older and poorer the preparation the feebler and shorter the

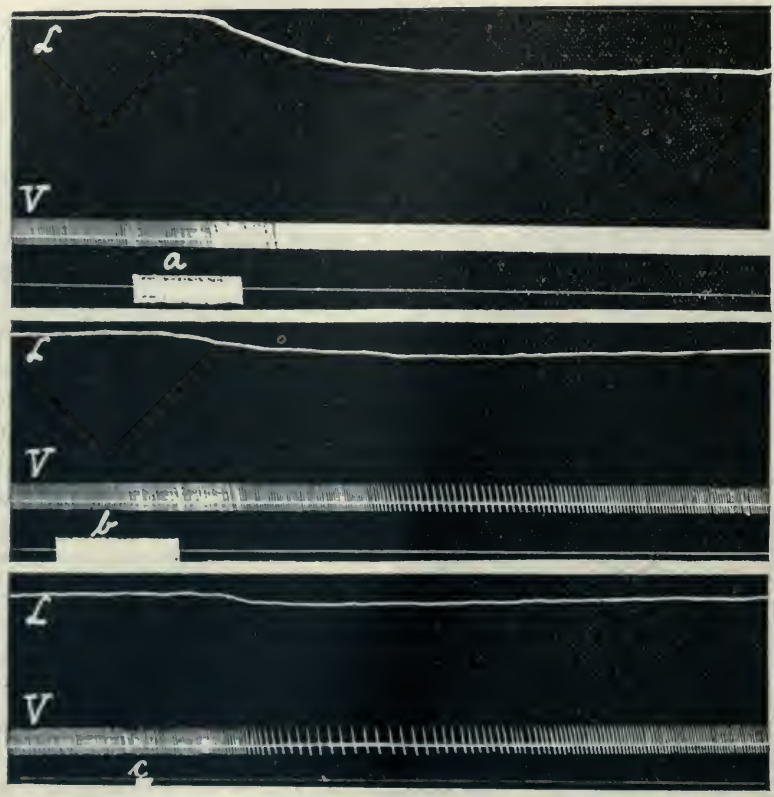

Fig. 16. Excised lung of the turtle. Perfused through pulmonary artery with Ringer solution + a small quantity of defibrinated turtle blood. Bronchus ligated, cannula for recording intrapulmonic pressure in tip of lung. *L*, record of lung tonus. *V*, venous outflow in drops. Addition of adrenalin to the perfusion solution close to the pulmonary artery, *a*, 0.1 cc.; *b*, 0.2 cc.; *c*, 0.5 cc. adrenalin (1:1000). Showing inhibition of lung tonus, and vasodilator and constrictor action of adrenalin on the pulmonary vessels.

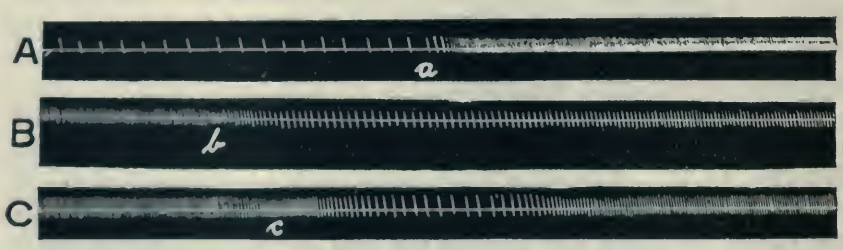

Fig. 17. Perfusion of isolated lung of turtle with Ringer + defibrinated turtle blood. *A*, lung vessels in strong tonus. *B* and *C*, lung vessels dilated. *a*, *b*, *c*, injection of $\frac{1}{10}$ cc. adrenalin (1:1000) into perfusion fluid into pulmonary artery. Showing opposite vasomotor action of the same concentration of adrenalin depending on the tonus of the vessels.

vasomotor response to epinephrin, and lungs perfused for 30 to 60 minutes with a Ringer-blood mixture and still giving good lung contractions in response to the tetanization of the vagus may fail to show any vasomotor effect even when enormous quantities (1 cc. 1:1000) of the drug are added to the perfusion mixture.

If the excised and perfused lung is in a state of marked tonus epinephrin in quantities that induce vasomotor action causes at the same time inhibition of the lung tonus (fig. 16).

On the basis of the prevailing theory of epinephrin vasomotor action, the above results show that the pulmonary blood vessels of the turtle are provided both with dilator and constrictor nerves. We have already adduced proofs of the presence of these nervous mechanisms independent of the epinephrin data, but the latter are interesting and may be interpreted as confirmatory. In the turtle as in the frog, epinephrin appears to exert its typical hemodynamic action on nerves that belong to the vagus, not to the sympathetic system.

Histamine as well as pituitrin causes pulmonary vasoconstriction and lung contraction (figs. 19, 20). The actions of these drugs on the two motor systems do not run parallel. In the case of the excised and perfused lung, at least, the blood vessels are more sensitive than is the lung musculature.

5. The action of atropine on the pulmonary vasomotor mechanism. Atropine in doses that paralyze the cardiac and the pulmonary vagi abolishes at the same time the vagus action on the lung blood vessels, just as in the frog. In fact, this drug may completely abolish the vasomotor lung action before the paralysis of the lung motor action (fig. 14 *C*).

The comments made in connection with this phenomenon in the frog need not be repeated here.

6. Lung vasomotor reflexes. Our attempts to secure vasomotor reflexes into the lung were not extensive. As in the frog, the vasomotor reflexes are not readily elicited, at least in animals prepared by our method. This may be due to a condition of prolonged "shock" of the pulmonary vasomotor center in the medulla or to the complexity of the peripheral nerve mechanism. It may be noted that motor reflexes into the stomach are, if anything, difficult to produce.

With the lung exposed and left with the circulation intact pulmonary vasomotor reflexes must be judged by direct inspection of the lung blood vessels. Slight changes in the caliber of the vessels cannot be detected by direct inspection. Perfusion of the lung in situ, with

the pulmonary vagus intact, permits more accurate records, but this method has the disadvantage of rapid failure of the vasomotor nerves. In figure 21 typical tracings secured by this method are reproduced, showing slight vasomotor action (dilator and constrictor) on stimulation of the posterior nares, and the central end of the sciatic nerve. Our few experiments on this phase indicate at least that pulmonary

Fig. 18. Record of perfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. Signal, introduction of $\frac{1}{20}$ cc. 1:1000 adrenalin in perfusion fluid near the pulmonary artery. Showing intense and prolonged vasoconstrictor action of the drug.

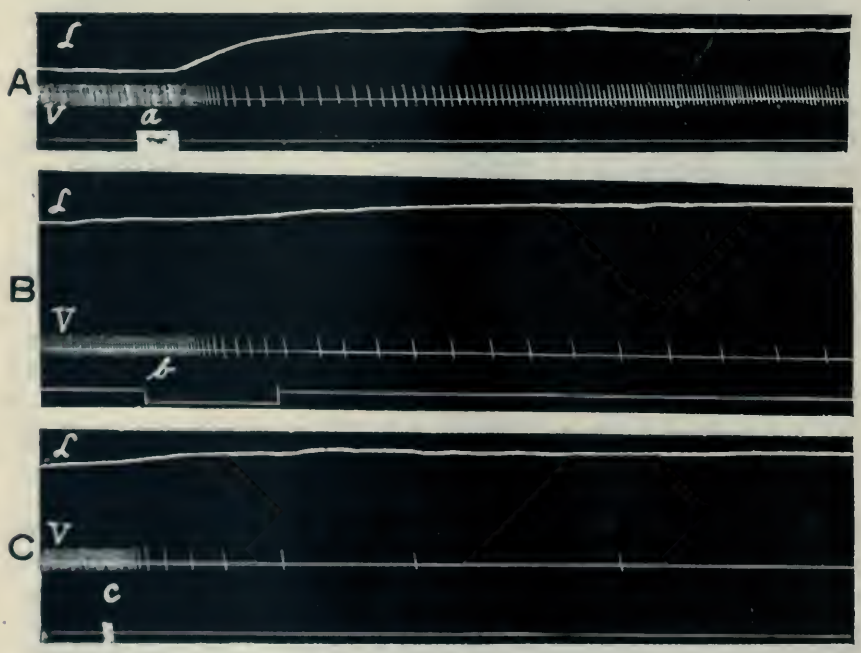

Fig. 19. Transfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of lung tonus (intrapulmonic pressure). *V*, rate of venous outflow (drops). Addition of histamine chloride to perfusion fluid near pulmonary artery, *a*, $\frac{1}{100}$ cc.; *b*, $\frac{1}{10}$ cc.; *c*, $\frac{2}{10}$ cc. 1:1000. Showing histamine contracture of the pulmonary blood vessels independent of the histamine tetany of the lung musculature.

vasomotor reflexes are much more difficult to elicit than the motor reflexes into the lung musculature.

7. The peripheral lung tonus and the secondary action of the vagus on this tonus. In our previous studies on lung automatism in the turtle,

Fig. 20. Record of perfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of lung tonus (intrapulmonic pressure). *V*, rate of venous outflow (drops). A, signal, addition of ½ cc.; B, signal, 1 cc. pituitrin to perfusion fluid near pulmonary artery. Showing pulmonary vasoconstrictor action of pituitrin independent of the contraction of the lung musculature.

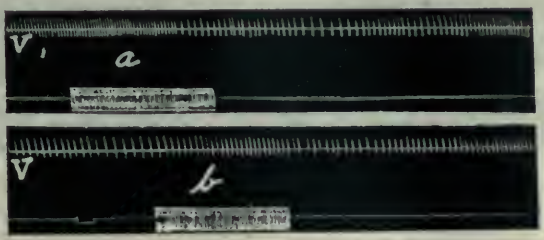

Fig. 21. Perfusion of the turtle lung (right) in situ. Animal decerebrated, curarized, and fixed dorsal side down. Anterior third of plastron removed to permit access to pulmonary vessels. Right vagus intact. Perfusion fluid, Ringer + defibrinated turtle blood. *V*, rate of venous outflow (drops). *a*, tetanization of central end of sciatic nerve; *b*, mechanical stimulation of the posterior nares. Indicating pulmonary vasomotor reflexes. (Record of the intrapulmonic pressure not obtained owing to mucus in the bronchus.)

we secured little or no evidence of the existence of inhibitory nerves to the lung motor tissues, and very little indication of a peripheral tonus independent of the motor fibers of the vagi and the activity of the cen-

tral nervous system. To be sure, a feeble lung rhythm was sometimes seen in the lungs after section of the vagi nerves.

In the present series we have frequently noticed a gradual increase in the lung tonus during perfusion with the Ringer-blood mixture, both of the excised lung and of the lung left in situ, and the vagus intact. On this tonus contraction there is always superimposed a feeble but

Fig. 22. Record of lung (left) tonus in a decerebrated and curarized turtle. Animal fixed dorsal side down. Platron removed. Left bronchus ligated. Cannula in tip of lung. Viscera removed from ventral surface of lung. Lung perfused through the pulmonary artery with Ringer + defibrinated turtle blood. *x*, section of left vagus in the neck. Showing a gradual increase in the lung tonus with indications of a peripheral tonus rhythm after section of the vagus. The intervals between the tracing sections A, B, C and D = about 10 minutes.

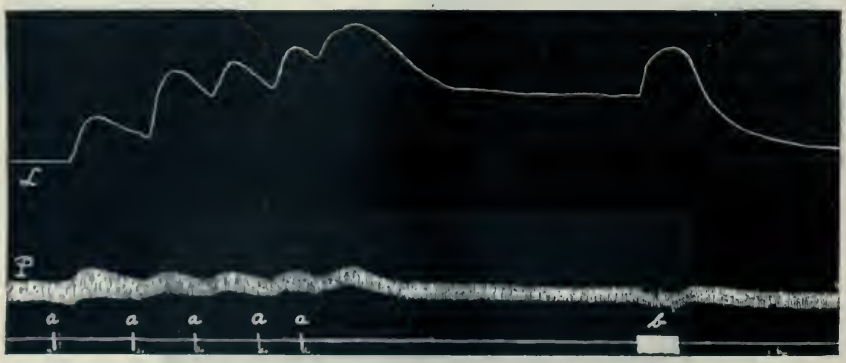

Fig. 23. Records of the blood pressure (right pulmonary artery) and left lung, *L*, tonus in a decerebrated and curarized turtle. Left lung isolated, except for pulmonary blood vessels and vagus. *a*, repeated injections into the jugular vein of $\frac{1}{10}$ cc. 1:100 histamine chloride; *b*, tetanization of left peripheral vagus. Showing inhibition of the histamine tetanus following the primary lung contraction on vagus stimulation.

distinct rhythm of varying rate. In the preparations where we perfused the lung *in situ* and with the vagus intact, it was noted at times that *the tonus rise began on section of the vagus nerve* (fig. 22). This seems to indicate that there are some inhibitory fibers to the lung motor tissues in the vagi nerves. The fact that epinephrin inhibits the tonus in the turtle lung points in the same direction, if one may accept the theory of exclusive nerve ending action of this drug.

We also wish to record another fact observed in this series that may be interpreted on this basis. If the perfused lung, excised or in situ, is in a state of considerable tonus, stimulation of the vagus still causes further contraction of the lung musculature, but following the stimulation the relaxation of the lung musculature does not stop at the tonus level existing prior to the stimulation but drops much lower, and this secondary tonus inhibition lasts for some time. This phenomenon occurs whether the lung tonus is spontaneous or induced by such drugs as histamine (fig. 14 *A*, fig. 23). We recognize that this fact is capable of several interpretations, such as depressor action of fatigue metabolites, etc. It may also be due to the action of inhibitory fibers in the vagus nerve outlasting the more direct and powerful action of the motor fibers.

SUMMARY AND CONCLUSIONS

Frogs: 1. Section of the vago-sympathetic nerves leads to dilatation of the lung arteries on the same side, parallel with the development of hypertonus of the lungs. This tonic constrictor action of the vago-sympathetic nerve on the lung arteries appears to be due to vagus fibers, as section of the sympathetic nerve by itself is without effect on the lung.

2. Stimulation of the peripheral end of the vago-sympathetic nerve induces contraction of the lung arteries on the same side. In good preparations this contraction is maximal. The pulmonary vasoconstriction is independent of the vagus action on the heart (elimination of cardiac vagus by curare, nicotine, direct perfusion of the lung, and excision of the heart) and on the lung motor tissues, as it may occur parallel with lung contraction, lung dilatation or no change in lung tonus. Stimulation of the peripheral end of the cervical sympathetic nerve has no effect on the tonus of the pulmonary arteries. Hence the vasoconstrictor fibers appear to belong to the vagus system.

3. In the lung perfused with a mixture of frog's blood and Ringer's solution minimal doses of epinephrin have a vasodilator action; larger doses, a vasoconstrictor action. The perfused lung deteriorates rapidly and these vascular responses to epinephrin occur only in very fresh preparations.

4. Histamine and pituitrin cause vasoconstriction in the lungs. The vascular motor tissues are more sensitive to these drugs than the lung motor tissues.

5. Atropine in doses that paralyze the cardiac vagus and the lung motor fibers in the vagus abolishes also the vasomotor action of the vagus on the pulmonary blood vessels.

6. Pulmonary vasoconstrictor reflexes have been obtained from the central vagus and the brachial nerves.

Turtles: 1. Section of the vagus causes dilatation of the pulmonary arteries on the same side. This is not due to cardiac and arterial blood pressure changes, because it occurs only in the lung on the side of the vagus section, and it occurs in the lung perfused *in situ*. Section of the cervical sympathetic nerve has no action on the tonus of the pulmonary blood vessels. The tonic vasoconstrictor action in the lungs appears therefore to be due to vagus fibers.

2. Periodic variations in the tonus of the pulmonary arteries may occur in the intact lung. These tonus variations may disappear on section of the vagus on the same side. A vasomotor rhythm of strictly peripheral origin has also been observed in the denervated lung, particularly under perfusion.

3. Stimulation of the peripheral end of the vagus nerve causes vasoconstriction in the lung of the same side. In a few preparations weak tetanization of the vagus caused vasodilatation in the lung. Stimulation of the cervical sympathetic nerve has no influence on the tonus of the pulmonary blood vessels.

The pulmonary vasomotor effects of tetanization of the vagus are independent of cardiac and arterial pressure factors (section of the cardiac vagus, direct perfusion of the lung), and are at least partly independent of the lung contraction induced by the stimulation.

4. In the lung perfused with a mixture of turtle's blood and Ringer's solution minimal doses of epinephrin cause pulmonary vasodilatation; larger doses, pulmonary vasoconstriction. The perfused lung deteriorates gradually, the vagus vasomotor action and the hemodynamic action of epinephrin disappear long before the lung motor action of the vagus.

5. The pulmonary vasomotor action of the vagus is abolished by doses of atropine that paralyze the cardiac and the lung motor vagi fibers.

6. Pulmonary vasoconstrictor reflexes have been observed from electrical stimulation of the central sciatic nerve and mechanical irritation of the posterior nares.

7. Histamine and pituitrin cause contraction of the pulmonary blood vessels. This action is independent of the contraction of the lung musculature induced by these drugs.

General: 1. We have been unable to obtain any pulmonary vasomotor action from the cervical sympathetic nerves in these animals. These are merely negative results, and we do not attach positive significance to them at present.

2. On the basis of the prevailing theory of epinephrin action, the pulmonary vasodilatation induced by minimal doses of epinephrin indicates vasodilator nerves to the lungs. In the turtle these vasodilator fibers appear to be included in the vagus; in the frog our work does not disclose the course of the vasodilator nerves.

3. Both in the frog and in the turtle atropine in pharmacological doses paralyzes the pulmonary vasomotor nerve mechanism. So far as we know this is the first clear demonstration of vasomotor paralysis by this drug. This fact appears to render questionable the work of previous investigators on the subject of pulmonary vasomotor control in the mammal where atropine was used to eliminate the cardiac factor.

4. It would seem that the pulmonary vasomotor reflexes (central and peripheral) must be taken into account in lung physiology and pathology. Drugs like epinephrin, histamine, atropine and pituitrin act parallel but independently on the vasomotor and lung motor mechanism.

5. The pulmonary vasomotor nervous mechanism is more readily fatigued and more quickly paralyzed by untoward physiological conditions than any other vasomotor mechanism known to us in the mammal. This may indicate a complex system of neurones on the pulmonary vasomotor pathway. This fact serves, at least in part, to explain past failures in the research on vasomotor control in the lungs. We are apparently dealing with a mechanism having the delicate physiological balance of complex reflexes. The animal must be in good condition and the lung fresh, particularly if perfusion methods are used. Comparisons by the perfusion method may not be made with the lung and the skeletal muscles (hind legs). The lungs appear to be as delicate organs as some glands (e.g., the pancreas). For the lung of the frog and the turtle Ringer's solution is a poison, not a physiological solution, as it is for the skeletal muscles and motor nerves. The delicacy of the mechanism may also explain the apparent difficulty in inducing pulmonary vasomotor reflexes under our experimental conditions.

6. Our evidence goes to show that the pulmonary vasoconstrictor nerves belong to the vagus system, that is, they are not nerve fibers that join the vagus from sympathetic connections outside the skull. We have also seen that epinephrin causes vasoconstriction in these

lungs. It is idle to speculate whether these facts demand a revision of the theory of epinephrin hemodynamics or a further analysis (anatomical and physiological) of the correlation of the vagus and the sympathetic systems.

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PARADOXICAL PUPIL DILATATION FOLLOWING LESIONS OF THE AFFERENT PATHS

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Received for publication January 24, 1921

In 1855 Budge (1) observed in a young rabbit, after section of the left cervical sympathetic nerve and of the branches distal (cepha'ad) to the right superior cervical ganglion, that the right was the smaller of the two pupils half an hour after the operation; whereas, 48 hours after the operation the right pupil was the larger of the two. In adult rabbits 24 hours after a similar operation he found the pupil larger on the side on which the post-ganglionic fibers had been cut. Following Budge many observers reported paradoxical effects in the pupil-dilator mechanism as the result of lesions in the course of the cervical sympathetic from the origin of its preganglionic fibers in the upper thoracic region of the spinal cord, to the ending of its post-ganglionic fibers in the region of the ciliary body and iris. The most noteworthy contributions on the subject came from Schiff (2), Kowalewsky (3), Braunstein (4), Lewandowsky (5), Langendorff (6), Anderson (7) and Meltzer and Auer (8).

Many observers noted the fact that after removal of one superior cervical ganglion, or section of one sympathetic nerve, the pupil on the side of operation under certain circumstances, e.g., asphyxia, excitement, injections of adrenalin, death, etc., became larger than its fellow. Because of the fact that the pupil on the side on which the efferent dilator path had been severed exhibited greater power of dilatation than its fellow, Langendorff (6) called the phenomenon paradoxical pupil dilatation, to the paradoxical phenomena already noted by other observers. Anderson (7), as the result of his investigations, added the paradoxical pupillary effects (constriction) consequent on section of the third cranial nerve or the equivalent of this, viz., removal of the ciliary ganglion or section of the short ciliary nerves. Elliott (9) found that paradoxical phenomena ensued in every organ supplied by the sympathetic system after section of the pre- or post-ganglionic fibers passing to that

organ. It should be noted that the paradoxical effects seen after lesions of the cervical sympathetic are not limited to the pupil but include widening of the *fissura palpebrarum*, withdrawal of the *membrana nictitans*, and bulging or *proptosis* of the eyeball. In the present studies attention was directed chiefly to alterations in the pupils and membranes. The width of the pupils, protrusion of the membranes, etc., were *estimated* where it is not expressly stated that actual measurements were taken.

None of the observers quoted, and none in the author's recollection, has made reference to the possibility of paradoxical effects consequent upon lesions of the afferent pathways, although it has long been known that stimulation of sensory nerves causes pupil dilatation. In the present studies after nerve section, etc., in cats, tests were made from time to time for paradoxical phenomena chiefly by injections of adrenalin into the jugular vein as first practised by Lewandowsky (5) and subsequently by Langley (10) and by Meltzer and Meltzer (11). Other methods of evoking paradoxical effects were occasionally employed such as asphyxiation, intravenous injections of pituitrin and of lactic acid, and finally instillations of adrenalin into the conjunctival sacs as practised by Meltzer and Auer (8). The condition of the pupils in death was carefully recorded as the death agony is a potent means of evoking paradoxical effects. When not otherwise stated adrenalin, etc., employed as an excitant of paradoxical effects, was administered intravenously. All operations were done under ether anesthesia. Before acceptance each animal was tested with adrenalin. The failure of conjunctival instillations of adrenalin to elicit frank paradoxical effects after sciatic section, etc., in instances in which paradoxical effects were readily elicited by adrenalin intravenously is of no significance since such instillations were often ineffective after cervical sympathetic section where adrenalin intravenously elicited paradoxical effects.

Section of one sciatic nerve. Numerous experiments were made in which one sciatic nerve was divided. In all instances paradoxical effects were readily evoked in the contralateral pupil by adrenalin. Description is confined to three animals with abstracted protocols.

In cat 1 well-marked paradoxical phenomena were evoked in the left eye 13 days after section of the right sciatic nerve. On the 2nd day after excision of the scar-area and suture of the divided nerve ends the paradoxical phenomena were still demonstrable in the left eye but had disappeared by the 4th day and were again demonstrable on the 13th day. On the 21st day after excision of the scar and suture

of the nerve ends the paradoxical phenomena had almost disappeared, being only manifest in the constriction period after adrenalin when the left pupil was larger than its fellow. On this day $1\frac{1}{2}$ inches of the distal segment of the divided nerve were removed and the gap bridged with catgut sutures. Twenty minutes later the paradoxical phenomena were about as immediately before the operation but were absent after that for 8 days and were demonstrable again 20 days after the operation. (Photograph 1.)

Fig. 1. Reappearance of paradoxical effects in the left eye after "freshening" the end of the proximal segment of the right sciatic nerve which had been sutured 41 days previously.

Fig. 2. Paradoxical effects in left eye on the 17th day after division of the right sciatic nerve and 7 minutes after section of both cervical sympathetic nerves.

Fig. 3. Reappearance of paradoxical effects in the right eye on 10th day after excision of the scar area in the left sciatic nerve which had been divided 18 days previously.

Fig. 4. Paradoxical effects in the left eye on the 12th day after injection of the right sciatic nerve with 60 per cent alcohol and 16 minutes after cord transection between roots Lii and iii.

On the 85th and 99th day (51st and 65th after excision of the distal segment of the divided sciatic, etc.), adrenalin failed to evoke paradoxical effects in the left eye. On the 99th day (65th after their insertion) the catgut strands were removed. On the 10th and 13th days after this last operation adrenalin failed to evoke appreciable paradoxical effects

Fig. 5. Paradoxical effects in the left eye evoked by ether anesthesia on the 37th day after section of posterior spinal roots Lvii to Sii on the right side.

Fig. 6. Paradoxical effects in the left eye evoked by adrenalin given intravenously on the 37th day after section of the posterior spinal nerve roots Lvii to Sii on the right side.

Fig. 7. Paradoxical effects evoked by adrenalin instilled into the conjunctival sac on the 37th day after section of the posterior nerve roots Lvii to Sii on the right side and 1 hour after transection of the cord between roots Lii and iii.

in the left eye. Evidently the removal of the catgut strands had little effect upon the paradoxical mechanism. Histological examination failed to show regenerating nerve fibers along the course of the catgut strands. One centimeter was now excised from the extremity of the proximal stump of the divided sciatic. On the 7th day after this last

operation adrenalin failed to evoke paradoxical effects. On the 9th day paradoxical effects were evoked but were easily exhausted since a lethal dose of lactic acid given a few minutes later failed to elicit them.

Protocol. Cat 1. Female, adult, non-pregnant, non-lactating

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
	a.m.		*mm.*	*mm.*	*mm.*	*mm.*
		July 12, 1919. Cut right sciatic nerve				
13th	9:44	Before adrenalin	5.0	4.0	2.0	2.0
	9:44½	Adrenalin ♏ii	8.0	11.0	0.0	0.0
		Paradoxical effects in left eye				
	10:30	Excised scar area and joined nerve ends with silk sutures				
26th		13th after excision of scar area				
		Paradoxical effects in left eye				
34th		21st after excision of scar area				
		Paradoxical phenomena waning in left eye				
		Excised 1½ inches of the distal segment and bridged space between the nerve ends with fine catgut after freshening end of proximal segment				
54th		20th after excision of distal segment, etc.				
	p.m.					
	2:03	Before adrenalin	2.5	2.5		
	2:03½	Adrenalin ♏ii	6.0	7.5	0.0	0.0
		Paradoxical effects in left eye: photograph 1				
99th		65th after excision of distal segment				
		Paradoxical effects absent in left eye				
	4:50	Removed catgut strands. No evidence of regenerating nerve fibers				
112th		13th after removal of catgut strands				
		Paradoxical effects absent in left eye				
		Excised 1 cm. from extremity of proximal segment				
123rd		9th after excision of 1 cm. from proximal segment				
	2:11½	Before adrenalin	7.0	5.0	2.0	3.0
	2:12	Adrenalin ♏ii	10.0	11.0	0.0	0.0
	2:12½	In constriction	8.0	9.0	1.0	2.5
		Trace of paradoxical effects in left eye				

In cat 2 slight paradoxical effects were elicited in the left eye 5 days after section of the right sciatic nerve. After excision of 1½ inches of the distal segment of the divided nerve paradoxical effects were elicited

Protocol. Cat 2. Young male about 4 months old

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		August 9, 1919. Cut right sciatic				
5th		Trace of paradoxical effects in left eye				
		Excised 1½ inches of distal segment				
17th		12th after excision of portion of distal segment				
	a.m.					
	9:05	Before adrenalin	3.5	4.0	3.0	3.0
	9:05½	Adrenalin ♎ii	8.0	9.0	3.0	3.0
		Paradoxical effects in left pupil but not in left membrane				
	9:36	Transected spinal cord between spinal roots Lii and iii				
	9:52	Before adrenalin	5.0	5.0	3.0	3.0
	9:52½	Adrenalin ♎ii	10.0	8.5	2.0	2.0
		No paradoxical effects in left eye				
	10:19	Before adrenalin	7.0	7.5	3.0	3.0
	10:19½	Adrenalin ♎ii	11.5	9.0	1.0	1.0
		No paradoxical effects in left eye				
	11:05	Transected spinal cord between roots Cv and vi				
	p.m.					
	12:08	Before adrenalin	2.5	2.5	1.0	1.0
	12:08½	Adrenalin ♎ii in 5 cc. of 5 per cent dextrose solution	10.0	11.0	1.0	1.0
		Paradoxical effects in left eye				
	12:41	Cut right and left sympathetic nerves in neck				
	12:46	Dextrose 5 cc. of 5 per cent solution intravenously; no effect on pupils				
	12:48	Adrenalin ♎ii in 5 cc. of 5 per cent dextrose solution	11.5	13.0	1.0	0.5
		Paradoxical effects in left eye: photograph 2				
	a.m.					
18th	2:30	Animal died				
	8:15	In death	8.5	9.0	6.0	5.5
		Trace of paradoxical effects in left eye: actual measurements				

only at times on the 3rd day (8th after primary nerve section) whereas on the 9th day (14th after the primary nerve section) paradoxical effects were consistently demonstrable in the left eye. On the 12th day (17th

after the primary nerve section) paradoxical effects were present in the left eye before spinal cord transection midway between the 2nd and 3rd lumbar roots. At different intervals ranging from 16 to 43 minutes after the cord transection adrenalin caused both pupils to dilate but the right was always larger than the left so that the paradoxical phenomena in the left eye had evidently been suspended temporarily as the result of the lumbar transection. Six minutes after spinal transection between roots Cv and vi adrenalin caused greater and more prompt dilatation in the right than in the left pupil although in the constriction period the left was for a time the larger of the two. Eleven minutes after the cervical transection adrenalin administered in 5 per cent dextrose solution evoked paradoxical effects in the left eye. Adrenalin administered without sugar solution 10 and 14 minutes later did not evoke paradoxical effects in the left eye. Half an hour later, however, or about 1 hour after the cervical transection, adrenalin in sugar solution evoked typical paradoxical phenomena in the left eye. The right and left cervical sympathetic nerves were then cut and 5 minutes later 5 cc. of sugar solution, given intravenously, had no effect on the pupils whereas adrenalin, given in sugar solution, evoked well-marked paradoxical phenomena in the left eye. (Photograph 2.) After these experiments the animal lived about 13 hours. Six hours after death traces of paradoxical phenomena were visible in the left eye. In assessing the value of these observations the reader is reminded that they were made *17 days* after the original nerve section; that is to say, at a period when among other things regenerative and compensatory changes had been established to a considerable extent in the spinal cord and dorsal root ganglia.

In cat 3 the cord was transversely hemisected on the right side at the level of root C ii. The nerve root was considerably damaged, as shown at autopsy. Immediately after the hemisection the right pupil was larger than the left. Three days after the operation the left pupil was much larger than the right. As time went on the difference in the size of the pupils diminished somewhat but the right remained persistently smaller by 2 mm. or more than the left. Adrenalin on the 34th, 54th and 76th days caused dilatation of both pupils but the right was always considerably smaller than the left. This was considered a good case to test the effects of section of the contralateral sciatic nerve upon the constricted pupil. Accordingly on the 77th day the *left* sciatic nerve was divided. On the 12th day after the nerve section although the right pupil under all ordinary circumstances was smaller than its fellow adrenalin evoked paradoxical dilatation in the right pupil which for a

short time was the larger of the two. By the 18th day adrenalin no longer evoked paradoxical phenomena in the right eye. The scar region of the divided nerve was now excised and a portion of fresh normal sciatic nerve from another cat sutured in place between the divided nerve ends and 10 days later, although the right pupil was considerably smaller than the left, adrenalin evoked well-marked paradoxical phenomena in the right pupil which became larger for a time than its fellow (photograph 3). On the 17th day after this last operation well-marked paradoxical effects could not be obtained by adrenalin nor by mechanical asphyxiation.

Protocol. · Cat 3. Male adult

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		May 11, 1919. Hemitransected spinal cord at level of root Cii on right side				
3rd			2.5	8.0	2.0	2.0
76th		Paradoxical effects absent in right eye				
		Cut left sciatic nerve				
88th		12th after section of left sciatic				
		Paradoxical effects in right eye				
94th		18th after section of left sciatic nerve				
		Paradoxical effects absent in right eye				
		Excised scar region of left sciatic and inserted transplant of fresh normal sciatic nerve				
104th		10th after insertion of transplant				
	a.m.					
	10:40	Before adrenalin	4.0	5.0	5.0	5.0
	10:40½	Adrenalin ♏ii	12.0	10.5		
	10:41	In constriction	10.0	8.0	1.5	1.0
		Paradoxical effects in right eye: photograph 3				
111th		17th after insertion of transplant				
		Paradoxical effects absent in right eye				
		Clamped trachea: pupils dilated but right always smaller than left	13.0	14.0	0.0	0.0
		Died				
		Autopsy. Scar of hemitransection involved right root Cii which was found damaged				

Alcoholic injection of one sciatic nerve. Owing to the popularity of alcoholic injections, made under the nerve sheath and sometimes into the nerve trunks for the relief of obstinate painful conditions resulting

from nerve injuries (causalgia, etc.), it was decided to test the effects of alcoholic injections into one sciatic nerve upon the pupils. The results were as consistent and striking as those obtained after sciatic section.

In cat 4 an injection of 60 per cent alcohol was made into the right sciatic nerve. Twelve days later paradoxical phenomena were in evidence in the left eye before and after cord transection between roots L ii and iii, and in death (photograph 4).

Protocol. Cat 4. Female about 3 months old

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		August 16, 1919. Injected ♏x of 60 per cent alcohol into the right sciatic nerve				
	a.m.					
12th	8:45	Before adrenalin	2.5	1.0	3.0	2.0
	8:45½	Adrenalin ♏ii Paradoxical effects in left eye	7.0	8.5	2.5	0.0
	9:30	Transected spinal cord between roots Lii and iii				
	9:40		5.0	5.0	1.0	0.5
	9:40½	Adrenalin ♏ii Paradoxical effects in left eye	8.0	10.0	1.0	1.0
	9:46	Gasping as dies Paradoxical effects in left eye; actual measurements of pupil: photograph 4	9.0	11.0	1.0	1.0

Similar results were obtained from another animal whose right sciatic nerve had been injected with 60 per cent alcohol.

Unilateral section of the lumbo-sacral posterior nerve roots. In cat 5 an attempt was made to sever the right posterior nerve roots L vi to S ii inclusive, without injuring the anterior roots and with the least possible disturbance of the cord and the left spinal nerve roots. The attempt was manifestly successful as the animal's posture and locomotion a few days after the operation could be regarded as very little impaired were it not for the fact that the toes of the right hind limb turned somewhat under the foot in plantar flexion so that the weight was borne on their dorsal aspect; on the 2nd and 12th days after the operation adrenalin failed to evoke paradoxical phenomena in the left eye. On the 30th day adrenalin failed to evoke paradoxical effects in the left

eye although the left pupil on this day, after each injection of adrenalin, began to dilate before its fellow. On the 31st day adrenalin applied locally to each conjunctival sac failed to evoke dilatation in the pupil of either eye. On the 37th day well-marked paradoxical effects appeared in the left eye incidental to ether anesthesia and after adrenalin intravenously, and applied locally to the conjunctival sac. Transection of the cord between roots L ii and iii caused momentary dilatation in both pupils but beyond this was without apparent effect upon the paradoxical phenomena which were as readily elicited after as before the operation (photographs 5, 6 and 7).

Protocol. Cat 5. Adult, male

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		September 5, 1919. Cut right spinal roots as follows: Lvi about ¾ and Lvii to Sii about ⅞ of whole distance across each root; no exposure or injury of roots on the left side				
	a.m.					
37th	9:50	Out of ether	1.5	1.5	3.0	3.0
	10:06	Under ether; wink reflex present but impaired	3.0	6.0	3.0	4.0
		Paradoxical effects in left eye: photograph 5				
	10:30½	Before adrenalin	2.5	6.0	3.0	3.0
	10:31	Adrenalin ♏ii in 1 cc. of 5 per cent dextrose solution	6.0	11.0	0.0	0.0
	10:33	In constriction	2.0	10.0	0.0	1.0
		Paradoxical effects in left eye: photograph 6				
37th	10:42	Transected spinal cord between roots Lii and iii				
	10:50	Before adrenalin	0.5	1.0	2.0	2.0
	10:53	Adrenalin ♏ii in 5 cc. of 5 per cent dextrose solution	8.0	9.0	0.0	0.0
	10:54	In constriction	4.0	9.0	1.0	1.0
		Paradoxical effects in left eye				
	11:04		1.0	2.0	2.5	2.0
	11:04½	Adrenalin ♏ii in each eye				
	11:53		1.0	9.0	1.0	1.5
		Marked paradoxical effects in left pupil; photograph 7				
	p.m.					
	4:20	Died				

In two other animals posterior nerve root section was done. The results were comparable to those just described though less striking quantitatively possibly because of slight injuries to the spinal cord and root ganglia.

DISCUSSION AND SUMMARY

After evulsion of one superior cervical ganglion intravenous injections or other paradoxical excitants do not elicit paradoxical effects before the expiration of 24 hours. Similarly after section of the sympathetic nerve in the neck paradoxical phenomena cannot as a rule be elicited before the expiration of from 5 to 7 days although under exceptional conditions they may be elicited as early as the 3rd day. The period of latency or "incubation" after section of one sciatic nerve varies from 8 to 12 days but the paradoxical phenomena may occasionally be elicited earlier, as in cat 2. The apparent prolongation of the "incubation" period in cat 5 after section of the posterior nerve roots was undoubtedly due to slight unavoidable injury of the cord or root ganglia at the time of operation. This induced on its own account pupillary phenomena which, for the time being, overshadowed the effects proper of the nerve root section. The well-known difficulty with which regeneration takes place in the posterior nerve roots after section explains the extraordinary persistence of the paradoxical phenomena as contrasted with their early appearance and disappearance after section or injury of one sciatic nerve. Following evulsion of one superior cervical ganglion paradoxical effects may be elicited in the corresponding eye for practically an indefinite period even though partial regeneration in the post-ganglionic paths may be demonstrated. After section of one cervical sympathetic nerve it is, however, easy to demonstrate that as regeneration takes place the paradoxical phenomena disappear, that is to say, in the course of a few weeks. The persistence of paradoxical phenomena so long after evulsion of one cervical ganglion is not a matter for wonder. Indeed when one considers the nature of the operation it becomes at once apparent that regeneration proceeds with difficulty and could hardly be expected ever to become complete. The condition here is analogous to what occurs after posterior nerve root section. After section or injury of one sciatic nerve the neurone bodies of the injured axones exhibit phenomena (axonal reaction phenomena, retrograde degeneration) indicative of suspension of function. These reach their maximum about

the 14th or 15th day, sooner or later, depending on circumstances, when the neurone bodies begin to exhibit changes indicating restoration of function. These changes take place in the proximal portions of the injured neurones, whether the divided nerve ends have been sutured or not, and there is evidence that at this stage and later on the regenerating neurones hyperfunction. It is noteworthy that after section of one sciatic nerve the disappearance of paradoxical effects coincides closely with the initiation of the disappearance of the axonal reaction phenomena. These facts taken in conjunction with the delayed disappearance of paradoxical phenomena after posterior nerve root section, and with the further fact that, in cat 1 after the disappearance of paradoxical phenomena these could be re-induced at will by further section or injury of the proximal segment of the divided sciatic, warrant the conclusion that after section or injury of one sciatic nerve the appearance and disappearance of paradoxical phenomena are conditioned respectively by the suspension and restoration of specific neural functioning in the related neurone bodies situated in the dorsal root ganglia.

Anderson (7), corroborating the prior finding of Lewandowsky (5), came to the conclusion that the mechanism of the paradoxical ocular phenomena after evulsion of one superior cervical ganglion, or section of one cervical sympathetic nerve, consists of increased irritability of the dilator pupillae muscle. Such increase of irritability of the effector mechanism of dilatation is the result of interruption of efferent impulses *via* the cervical sympathetic. After sciatic section or injury there occurs a similar failure of outflow of impulses to the dilator mechanism over the cervical sympathetic nerve though less in degree than in the case of ganglionectomy or section of the cervical sympathetic. The conclusion seems warranted that in the waking state afferent impulses coming from the periphery, or at least from that portion of it supplied by the sciatic nerves, are immediate factors in conditioning the outflow of impulses from the upper thoracic cord segments which govern pupil dilatation through the cervical sympathetic nerve. There is, moreover, some evidence pointing to the probability that each sciatic nerve sends impulses to the upper thoracic segments of both sides (lower cilio-spinal center of Budge) although the relation in this respect is mainly a crossed one.

The temporary disappearance of paradoxical effects in cat 2 immediately after spinal cord transection has its counterpart in the ease with which exhaustion occurs in the paradoxical mechanism at certain times. Thus after ganglionectomy or sympathetic section, toward the end of

the incubation period, or, in the case of sympathetic section, in the regeneration period just before the paradoxical phenomena disappear, repeated injections of adrenalin may fail to evoke paradoxical effects unless a period of rest be allowed to intervene. Here the effect of dextrose solution in restoring the exhausted effector mechanism is significant. Persistence of the possibility of evoking paradoxical effects after cervical and lumbar cord transection as well as after section of both cervical sympathetics, is further evidence that the paradoxical pupillary phenomena observed after section or injury of one sciatic nerve are identical in kind with the paradoxical phenomena observed after ganglionectomy or section of the cervical sympathetic nerve. This conclusion is further supported by observations made upon cat 5 in which, after section of the lumbo-sacral posterior nerve roots, adrenalin applied locally to the conjunctival sac induced marked paradoxical phenomena in the contralateral eye.

CONCLUSIONS

1. After section or injury of one sciatic nerve the appearance and disappearance of paradoxical phenomena in the contralateral pupil are conditioned respectively by the suspension and restoration of specific neural functioning in the injured neurones of the proximal segment and more particularly in the neurone bodies situated in the dorsal root ganglia.

2. In the waking state afferent impulses from the periphery, passing inward over each sciatic nerve, impinge upon the motor neurones situated in the lateral horns of the upper thoracic segments (lower cilio-spinal center of Budge) conditioning reflexly the outflow of impulses that reach the pupil-dilator mechanism *via* the cervical sympathetic nerves.

3. The impingement of afferent sciatic impulses upon the upper thoracic cord segments is probably bilateral although the maximal effect is exerted upon the segments of contralateral side.

4. The paradoxical pupillary effects observed after section or alcoholic injection of one sciatic nerve are identical in kind with the paradoxical phenomena observed after removal of one superior cervical ganglion or after section of one cervical sympathetic nerve.

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FATIGUE IN FROG MUSCLE WHEN IMMERSED IN VARIOUS CONCENTRATIONS OF LIPOID-SOLVENTS; ESPECIALLY THE HIGHER ALCOHOLS

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Received for publication February 1, 1921

In a recent paper (1) experiments were cited which indicate that developing sea-urchin eggs when subjected to suitable concentrations of various lipoid-soluble substances, i.e., the higher alcohols, show unmistakable rhythms of susceptibility and resistance according to the phase of physiological activity at the specific time of treatment. Such observations constitute additional evidence that a very intimate relation or correlation exists between the general physiological condition of the egg and the physical state of its plasma-membrane. The present paper is a preliminary report of experiments conducted in the light of recent advances to analyze the effects of various concentrations of the alcohols upon the resulting fatigue curves of excised frog muscles so immersed. The bearing such a study has on the theoretical and practical aspects of responses is apparent when one recalls that in any protoplasmic system, an increased (sensitization) or decreased irritability or spontaneous activity (anesthesia) may be brought about by the conditions of concentration, temperature, and the physiological state of the system.

In the case of substances, in proper concentrations, producing increased irritability, numerous examples might be cited both in plants and animals. It is well known that general nervous excitability is increased by weak doses of ether, alcohol and other active substances. Rhythmical activity such as that which takes place in cilia, or the heart beat, etc., is increased in weak solutions of alcohol and other narcotics. Carlson (2) has demonstrated that the nerve-cells controlling the heart beat of Limulus are induced to faster rhythmical action in weak solutions of alcohol, chloral hydrate, chloretone and chloroform. In experiments by Tashiro and Adams (3) similar responses in excitability in the nerve

and its output of carbon dioxide were noted when treated with low concentrations of urethane and chloral hydrate. In muscle-nerve physiology, the phenomenon of "treppe" exhibited by contracting muscle is probably due, as has been shown by Lee (4), to the stimulating action of small quantities of fatigue substances, which in higher concentrations decrease irritability. On the plant side numerous practical uses have been made of the fact that many depressant substances when administered in low concentration increase rate of growth; thus ether has been used in "forcing" plant growth by those interested in commercial horticulture.

Other striking effects of narcosis have been recorded in plants when treated with weak solutions of chloroform and ether on the side of oxygen consumption. (Tashiro and Adams cite observations of Kosinski showing that respiration in yeast cells increased in presence of 0.5 per cent ether; 5 per cent reduced respiration one-half, while 7 per cent almost stops it (4).) Rotation in plant cells has been observed within the protoplasm during the early stages of ether and chloroform narcosis, and it is recorded that the irritability of certain sensitive plants is heightened in traces of ether.

Loeb, Lillie, Torrey, Moore and others have observed striking behavior activities induced in various organisms when treated with the proper concentrations of certain anesthetics. Thus Loeb was able to produce a positive heliotropic response in Daphniae when subjected to weak solutions of alcohol and ether in strengths that vary from a third to a half of those required for anesthesia. Lillie in experiments with the marine annelid larvae (Arenicola) found that he could change the behavior from a normally positive to a negative heliotropism in various weak anesthetic substances.

On the other hand and opposite to sensitization is the phenomenon of reversible decrease in irritability or responsiveness which is anesthesia, and the vital processes that are subject to such an arrest are numerable and varied, and may be brought about in a number of different ways, i.e., mechanical, thermal, electrical or chemical. In discussing the theory of anesthesia, Lillie (7) lists a few of the vital processes so effected as follows: They include amoeboid movements; protoplasmic rotation in plant cells; all processes depending on response to stimulation, like muscular contraction and stimulation and conduction in nerve; automatic rhythmical activities like the heart beat or the motion of cilia or spermatozoa; cell-division; the artificial initiation of development in unfertilized eggs; various fermentative and oxidative processes; light-

production, e.g., by luminous bacteria; typical metabolic processes like the assimilation of carbon dioxide by plants; growth processes in plants and animals, and developmental processes dependent on growth and cell division. It is of especial significance to note that processes depending upon growth and development are included in the list, and when anesthesia is administered during proper progressive developmental stages, far-reaching consequences may result. Thus abnormalities of growth and development as well as changes in irritability may be produced under the influence of anesthesia, as Stockard (5) and McClendon (6) have shown in the production of cyclopia in developing fish eggs, and other developmental defects produced by alcohol in the case of mammals as later shown by Stockard.

It is beyond the scope and purpose of this brief introductory review of facts concerned with responses of the vital processes to attempt to discuss the cause or causes of observed phenomena, but it seems logical to infer that like manifestations of ordinary stimulation, they are in some way intimately dependent on surface-changes of the plasma-membrane. The question as to just how these surface-changes are effected is a critical one, and one that needs more careful research. Lillie (7) in his discussion of the theory of anesthesia has thrown some light on the problem and suggests that it is highly probable that the whole processes of sensitization and anesthesia are akin to the processes of stimulation. They depend upon local initiation of the excitation state which is in turn qualified by semipermeability, electrical polarization and specific metabolic activity. Hence altering the physical and chemical conditions of the membrane alters the whole physiological process. With this brief discussion, we may proceed to the consideration of the mode of experimentation described.

Methods and apparatus. The experiments about to be described were performed intermittently between April and December of the past year, the frogs being obtained in five dozen lots from a supply house in Chicago, the batches consisting for the most part of the common or leopard frog, Rana pipiens *Schreber*, sometimes called Rana virescens *Kalm.* After making all due mechanical arrangements, the specimen was killed by pithing, the brain and spinal cord destroyed, and the gastrocnemius muscle removed and placed immediately in the glass cylinder of a Harvard type muscle warmer, arranged and mounted in such a way as to allow easy manipulation in pouring solutions of desired strengths, and so connected to the inductorium and the light muscle lever as to allow the transcribing of a record on the kymograph when the muscle was

stimulated by the intermittent induction shocks. Immediately above the muscle lever was mounted a Deprez double electric signal marker, the upper marker being connected through wires with a Harvard type chronometer beating off intervals of a half-minute; the lower being connected through wires with the primary circuit through a Harvard type vibrating interrupter beating seconds (a make and break during the second so that the muscle actually received through the inductorium two stimuli per second). The current used through wires was from electric storage battery, type "D," cells maintained approximately at full charge (1.225 s. g.) delivering a voltage to the inductorium of about 2 per cell. Usually two cells were used to impel the induction coil of the interrupter, with one cell furnishing the current to the inductorium and the muscle which of course was connected to the inductorium through the terminals of the secondary coil. The secondary coil in all experiments was placed over the primary so that it was 2 cm. from its fully closed position, thus delivering to the muscle practically its maximal induction. After extended preliminary experimentation it was found that about the optimum mechanical advantage in terms of leverage for the muscle lever consisted of having the muscle attachment 8 mm. from the pivotal fulcrum opposed by a 30 gram weight placed 14 mm. on the opposite side, the entire length of the writing arm being 150 mm. from the pivot. The kymograph used in recording was of the modified Harvard slow driving, long paper type, so provided with a fan that it made one revolution in about 17 minutes. All records shown in the subjoined plates were made with the drum revolving at this uniform speed so that from this factor they are comparable.

During the experimentations care has been exercised to keep the various mechanical and thermal conditions as constant as possible; the temperature has not been allowed to fluctuate more than 2 degrees at any time, usually the room temperature being kept at approximately 21°C. during all the experiments. The various concentrations of fluids used were made up in advance according to computed volumes per cent, and placed in small, cork-stoppered flasks of 150 cc. capacity, so that at the time of experimentation they were approximately that of room temperature. The manner of attachment of the muscle to the electrodes was carefully observed, and consisted of inserting the needle in the tendon achilles just at the junction of the fascia of the numerous muscle bundles for the lower contact; the upper end of the muscle being made secure by piercing the tendonous fascia at the knee joint between the distal end of the femur and the proximal end of the tibia. This

precaution to make secure the electrodes was found to be imperative since otherwise the inertia of the falling weight on the writing lever opposing the muscle would invariably alter the elasticity of the muscle and complicate the curves, especially in the initial excursions of the lever.

By closing the short ciruit bar on the key, the interrupter in the primary circuit activated intermittently the signal magnet and at the same time sent the make and break shocks to the muscle through the inductorium. It is assumed that by keeping constant the relative positions of the primary and the secondary coils, the resulting successive induced shocks which reach the muscle will be approximately of the same strength throughout any series of experiments. This factor, of course, is a very important one since the responses of skeletal muscles when supplied with gradations of current are markedly affected in a number of its phases, especially fatigue, as has been recently shown by Pratt (8). Assuming the factors just mentioned to be fairly constant throughout the series of experiments to be described, there is yet another variable not easily controlled, that of metabolic variability in the individual muscles. Selection as to equality in size and weight of the experimental muscles has been the only available criterion on this point. From comparative uniformity of the resulting curves obtained on repeated trials it would seem that this criterion could be relied upon within the limits of experimental procedure.

The alcohols used throughout the experiments were obtained from different sources; the methyl, ethyl and n-butyl were redistilled in the organic laboratory here at the college; the n-propyl was obtained from Merck; n-amyl, nonyl, decyl were obtained from the Eastman Kodak Laboratories, the last two named being put on the market as "technical" and were not used in this series of experiments. The secondary octyl was purified by the Eastman Kodak Company while its isomere capryl was "practical" and was redistilled here in the organic laboratory. Eastman's "technical" heptyl alcohol was used but for the most part records with this alcohol were unsatisfactory, probably because of its oily nature.

Experimentation. Ranges in suitable concentrations of the alcohols vary widely according to the specifically desired physiological result. For example, Overton in his study of narcosis of tadpoles found the range to lie between 0.57 mol per L. for methyl to 0.0004 mol per L. for octyl. Contrasted to these concentrations are observations by Fühner and Neubauer in producing hemolysis where the range varied from 7.34

mols per L. for methyl, to 0.004 for octyl, and Vernon's range in the destruction of indophenol oxidase was even higher in the first four members of the series that he tried, being 10.5 to 14 mols per L. for methyl to 0.032 to 0.9 mol for butyl. These last figures correspond very closely to those found to apply to isocapillary solutions, e.g., from 14.0 mols per L. for methyl to 0.14 mol for amyl. On this basis it was necessary to compute ranges of concentrations over rather wide limits, and to select therefrom those concentrations that promised to produce the desired physiological effects, i.e., bearing in mind that solutions of strengths above the optimum concentration would likely be too toxic and would result in depression or inhibition (anesthesia) of the muscular response, and on the other hand those below would probably have a stimulating (sensitization) effect.

TABLE 1

ALCOHOLS	CONCENTRATIONS OF ALCOHOLS				
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
Methyl	Strong	50	29.1	20.8	12.4
Ethyl	95.0	50	17.2	9.1	7.4
N-Propyl	Strong	Saturated	13.3	5.9	3.7
N-Butyl	Strong	Saturated	4.7	3.4	1.7
N-Amyl	Strong	Saturated	1.1	0.5	0.2
Heptyl	Strong	Saturated	1.6	0.7	0.4
S-Octyl	Strong	Saturated	0.62	0.29	0.15
Capryl	Strong	Saturated	0.50	0.15	0.05

The alcohols were diluted with distilled water. Under the conditions of experiments the muscles when immersed in physiological salt solution gave the typical initial contracture (treppe) phase for four or five strokes of the lever, followed by a decided relaxation phase longer in duration during which time an increased responsiveness was apparent. The second contracture phase develops regularly after the space of about a half a minute, and the muscle shortens in such a way to show a fall in the general contour of the top of the curve, accompanied by a gradual rise in the lower portion of the record. A typical record of this kind is shown in figure 12. A similar series of phases occurs when the muscle is stimulated in air, there being in this case no impediment in resistance to overcome by the muscle in its contraction, the resulting curve is comparatively larger, as shown in figure 6. The superimposed lighter curve in this case results from the "make shocks" while the larger and darker area is caused by the more intense "break shocks."

The mean time necessary to fatigue a muscle either in physiological salt solution or in air was found to be about 4 minutes, as recorded by the chronometer beating half-minutes, basing the calculation arbitrarily upon the point at which the lower margin of the second contracture phase begins its final descent.

Methyl alcohol. Strong methyl alcohol is markedly stimulating during the first few induced contractions (perhaps a dozen strokes of the lever), but its toxic effect is immediately noticeable and the progress of the second contracture is rapidly completed, the whole taking place in less than 2 minutes. A typical curve using this alcohol is shown in figure 1.

Methyl alcohol in strength of 50 vol. per cent (fig. 2) produces a rather uniform curve. The initial contracture phase is noticeably lacking, with an accompanying relaxation phase apparent as indicated by a drop in the excursion of the writing point. The second contracture phase is similar in form and duration to that produced by alcohol of great strength, fatigue resulting in this case in about $1\frac{3}{4}$ minutes.

Alcohol of 29.1 vol. per cent, as typically shown in figure 3, is decidedly stimulating during the initial contractions, but produces rapid fatigue once the secondary contracture phase is entered upon, the fatigue process lasting only $1\frac{1}{2}$ minutes.

Of all the concentrations tried with this alcohol it would seem from the records obtained that 20.8 vol. per cent gives the best combination of initial stimulation followed by rapid fatigue indicating toxicity and alcohol of approximately this concentration may perhaps be considered as about on the border, possessing favorable penetrating qualities. The muscle reached its final contracture phase in about $2\frac{1}{4}$ minutes and produces an interesting curve as shown in figure 4.

Methyl alcohol of 12.4 vol. per cent is obviously below optimum concentration to produce rapid fatigue. It, like the higher grades of this alcohol, stimulates noticeably during the initial contractions, proceeds to assume contracture proportions. A typical curve produced by muscles thus subjected is shown in figure 5, and from comparison is similar to one produced in normal salt solution (fig. 12).

Ethyl alcohol. Strong ethyl alcohol has a surprising stimulating effect upon muscles undergoing the fatigue process, so that the onset of the second contracture phase is very slow and remarkably gradual. The treppe effect brought about is perhaps more conspicuous here than in any other cases tried. Once the final contracture phase is produced, however, it is maintained for a long time with scarcely any decline ap-

Plate 1. *1.* Typical fatigue curve resulting from stimulation of muscle immersed in strong methyl alcohol. Note the initial relaxation phase is almost lacking, and the temporary stimulating effect as evidenced by the height of the first few contractions.

2. Fatigue curve of muscle immersed in 50 vol. per cent methyl alcohol. The early relaxation phase is here beginning to be conspicuous and is somewhat prolonged.

3. Typical curve when immersed in 29.1 vol. per cent methyl alcohol. Relaxation phase more pronounced, conspicuous treppe, but rather rapid onset of secondary contracture.

4. Curve resulting on immersion in 20.8 vol. per cent methyl alcohol. This concentration perhaps may be regarded as lying in a relative range of concentration where phases are well balanced in modification. A rather prolonged initial relaxation with long constant strokes, a uniform secondary contracture terminating in a reversible decline toward the end.

5. Curve resulting from immersion in the weakest alcohol used, 12.4 vol. per cent. Somewhat stimulating as evidenced by the early relaxation phase, the height of the excursions and the duration of upper margin of the plateau of secondary contracture.

6. Comparative curve resulting from stimulating muscle in air. Note the height of excursions, the initial relaxation phase, and the gradual and uniform development of secondary contracture. The whiter area inside is caused by the less intense "make" shocks.

7. Typical strong ethyl alcohol curve. Practically no initial relaxation phase, gradual onset of secondary contraction, a conspicuous treppe and a sustained contracture plateau.

8. Typical curve using 50 vol. per cent ethyl alcohol. Phases in marked contrast to proceeding, initial relaxation, rapidly developing secondary with maintained plateau becoming reversible.

9. Typical curve using 17.2 vol. per cent ethyl alcohol. Stimulating with modified toxic effect, note the rather prolonged initial relaxation followed by rapidly developing secondary with sharply declining plateau.

10. Typical curve using 9.1 vol. per cent ethyl alcohol. Stimulating with more slowly toxic effect. Gradual onset of secondary contracture and maintenance of plateau and reversible decline.

11. Typical curve using 7.4 vol. per cent ethyl alcohol. Not greatly different from preceding.

12. Typical curve obtained under same experimental conditions as all the others but in physiological salt solution, 0.7 vol. per cent. Note the initial relaxation phase accompanied by treppe at the top, the gradual onset of secondary contracture, and a slowly reversible decline, with maintained gradual inclined plateau.

13. Characteristic curve resulting from immersion in strong propyl alcohol. Primary contracture very evident with no reversal to relaxation whatsoever, immediate onset of secondary contracture, terminating in irreversible plateau.

14. Typical curve using saturated solution of propyl alcohol. A slight assumption of initial relaxation, no treppe, uniform plateau almost irreversible.

15, 16, 17. Typical curves using 13.5, 5.9 and 3.7 vol. per cent propyl alcohol. First two maintaining almost an irreversible plateau, the last showing a rapid decline.

Plate 2. *18.* Typical curve in strong butyl alcohol. Practical absence of initial relaxation phase, rapid onset of secondary contracture with maintained final plateau.

19. A suggestion of initial relaxation phase in saturated solution of butyl alcohol, with maintained horizontal plateau which is slowly reversible.

20, 21, 22. Curves resulting on immersion in 1.7, 3.4 and 4.7 vol. per cent butyl alcohol, respectively. By a mistake the order in strengths was reversed in the labeling. *22* is the strongest (4.7), *20* the weakest (1.7). Characteristic in reversible decline.

23. Typical curve in strong amyl. Note similar contours to those obtained with propyl and butyl alcohols of equal strengths.

Plate 1

Plate 2

24. Typical curve obtained in saturated solution of amyl alcohol. Comparatively similar to equivalent strength of propyl and butyl alcohols.

25, *26*, *27*. Curves produced in solutions of 1.1, 0.5 and 0.2 vol. per cent amyl alcohol, respectively. Characteristically different from those of similar strengths of propyl and butyl, and more closely resembling those of weak solution of methyl alcohol, *3*, *4* and *5*.

28. Curve resulting from immersion in secondary octyl alcohol and in sharp contrast to those in strong solutions of its predecessors, propyl, butyl and amyl alcohols. Notice an initial relaxation phase which occurs nowhere else in curves of strong alcohols, and almost as extensive as that produced in salt solution, *12*, in fact, in all its details it is almost a duplication of curves produced in salt solution.

29. Saturated solution of secondary octyl alcohol. It contrasts sharply with curves obtained from similar concentrations of propyl, butyl and amyl alcohols, and its nearest simile is that produced by comparative strength of ethyl alcohol, *8*.

30, *31*, *32*. Curves of secondary octyl alcohol of weak strengths, 0.62, 0.29 and 0.15 vol. per cent, respectively. These as can readily be seen are easily comparable to those obtained from similar strengths of butyl, and to a less degree of propyl alcohols.

parent. Muscles in this strengh fatigue quite as slowly as those submitted to physiological salt solution. Figure 7 shows a typical record obtained with strong ethyl alcohol. There is little initial relaxation apparent such as develops in concentrations of lower strengths.

Ethyl alcohol of 50 vol. per cent is remarkable in that it produces a sustained relaxation phase of uniform responsiveness as evidenced by the plateau form of the record (fig. 8), accompanied by a rather uniform base line. Once the fatigue is brought about, the second contraction phase appears rapidly as evidenced by the uniform ascent of the lower margin of the described curve. Contracture, however, is not prolonged as in strong alcohol, but begins immediately and is regular as in the case of normal fatigue.

The relative effects of subjecting muscles to the three lower concentrations of ethyl alcohol, namely 17.2, 9.1 and 7.4 vol. per cent, may best be seen by referring to typical curves shown respectively in figures 9, 10 and 11. That an increase in sensitization is caused by a decrease in concentration is strikingly apparent here. The highest of these three concentrations brings about almost immediately the onset of secondary contracture, so that the upper margin of the curve drops regularly while the lower margin has an accompanying uniform rapid rise, the whole fatigue process taking only $1\frac{1}{2}$ minutes to complete. Contrasted to this type of curve are those of lower concentrations, where the secondary contracture phase is gradually induced and maintained at a relatively higher and more uniformly regular level. In both of these the time necessary to fatigue is better than $2\frac{1}{2}$ minutes, and the increased responsiveness of the muscle is evidenced by the increased height of the recording lever. Once initiated, the decline in the secondary relaxation is rapid in all three cases, which is in marked contrast to curves produced by practically all concentrations of propyl or butyl alcohols (see figs. 13 to 19 inclusive) or in the case of strong methyl and ethyl alcohols (figs. 1 and 7). This point, it would seem, is an important one in the analysis, since it implies that once the stimulating effect has run its course, changes are brought about in the muscle which in turn now reverse the process and something akin to anesthesia ensues so that the relaxation is induced as rapidly as contracture was at first effected.

Propyl alcohol. Propyl alcohol in all ranges of concentrations except one (3.7 vol. per cent, fig. 17) gives striking and characteristic fatigue curves as may be seen by referring to figures 13 to 17. On being immersed the muscle immediately begins to shorten and this is accompanied by an increased sensitization in most of the concentrations tried.

This tendency to immediate shortening is especially noticeable in strong and saturated solutions, but is more or less conspicuous in the lower concentrations. In strong propyl alcohol there is only a suggestion of the treppe, the noticeable phase being the uniform secondary contracture which is maintained at a relatively high level (fig. 13). Saturated propyl forms a plateau with accompanying contracture (fig. 14) with maintained high contracture phase. In strengths 13.3 and 5.9 vol. per cent the resulting curves are very similar to one another as shown in figures 15 and 16, respectively. In the lowest concentration tried, 3.7 vol. per cent, the muscle fatigues uniformly and quickly, but after the completion of the second contraction phase it goes into relaxation rapidly so that the resulting curve (fig. 17) is in this respect exceptional to the other concentration of this alcohol, but comparable to the lower strengths of ethyl alcohol noted above.

Butyl alcohol. Strong and saturated solutions of this alcohol give curves (figs. 18 and 19) which are typical and which are comparable with similar strengths of butyl alcohol just described. Contracture starts almost immediately on being stimulated and is maintained at a remarkably uniform rate with almost no relaxation phase at the end of the fatigue cycle. The three solutions of weaker strength of this alcohol tried, namely, 4.7 (fig. 22), 3.4 (fig. 21) and 1.7 (fig. 20) vol. per cent gave typical curves similar to each other and to the weakest concentrations of ethyl and propyl alcohols. This would seem to indicate that the differences in range as computed were not sufficient to give decided differences in effective physiological responses sufficient to analyze. All tend to have a definite comparable initial relaxation phase as indicated by the drop in the writing point in the early progress of the curve, and all on the completion of the second contracture phase have a rather rapid onset of secondary relaxation as indicated by the abrupt descent at the terminal portion of the curves. It is very evident, too, that these concentrations have an exhaustive stimulating effect since fatigue once started proceeds rapidly, and that in this respect they are more potent than the three low grades of either methyl and ethyl alcohols and even more so than comparable toxic strengths of its predecessor, propyl alcohol.

Amyl alcohol. In strong and saturated concentrations amyl alcohol gives curves similar to those of comparable strengths of both propyl and butyl alcohols and decidedly in contrast to comparable strengths of methyl and ethyl alcohols. With strong amyl alcohol the first contracture phase is produced immediately on being stimulated, as shown

in figure 23, and proceeds gradually to produce the secondary contracture (see fig. 13 for comparison with propyl alcohol). The plateau of secondary contracture is with this alcohol more pronounced and extensive than is the case with propyl or butyl alcohols. The three lower concentrations used, 1.1, 0.5 and 0.2 vol. per cent, strange to say, give curves (figs. 25, 26 and 27, respectively) which in all details more closely resemble the three comparable strengths of methyl alcohol than any other concentrations of its predecessors (see figs. 3, 4 and 5 in comparison).

Hexyl and heptyl alcohols. No hexyl alcohol was available at the time of experimentation so that no records were obtained. Heptyl alcohol was at hand and its effects on fatigue in different concentrations somewhat explored but due to incompleteness of records no comparisons of value can be made at this time.

Octyl alcohol. Two isomeric solutions of this alcohol were tried, a secondary octyl from a purification process of the Eastman Kodak Company, and a so-called capryl alcohol from the same source, redistilled here. Little difference physiologically could be seen in using either in similar concentrations. Both give curves (figs. 28 and 29) in strong and saturated solutions which are remarkable in their similarity, not to their immediate predecessors of equal strengths, but to ethyl alcohol. The three lower concentrations, however, give curves (figs. 30, 31 and 32) which are fairly comparable to similar concentrations of butyl alcohol, with perhaps a less marked similarity to the weakest strength of ethyl and propyl alcohols. These considerations when taken together with certain other data seem to point to the fact that octyl alcohol in various concentrations does not exert as striking penetrating qualities as butyl, propyl or amyl alcohols. From incomplete records obtained in use of heptyl it would seem that this alcohol also is in a similar category.

SUMMARY

Making use of proper laboratory apparatus herein described in which experimental conditions may be kept reasonably constant, records were obtained in the development of fatigue in the gastrocnemius muscle of the frog while being subjected during its stimulation to certain computed concentrations of various alcohols.

The ranges of concentrations explored may be briefly tabulated; strong and saturated solutions of methyl, ethyl, propyl, butyl, amyl, heptyl, octyl and capryl, with computed gradations in three series of

each, varying from 29.1 methyl, to 0.62 vol. per cent octyl; 20.8 vol. per cent methyl, to 0.29 vol. per cent octyl; and 12.4 methyl to 0.15 vol. per cent octyl, respectively.

On comparative analysis of the various phases of these curves certain inferences can be drawn as to penetration and its resulting effects on the muscle of the different alcoholic concentrations used, both as to stimulation or sensitization and inhibition or anesthetic effects. Strong concentrations in general give remarkably uniform modifications in phases of contraction, especially in producing immediate contracture which merges without interruption into irreversible secondary contracture. Certain weak solutions in general are markedly stimulating as evidenced by initial and somewhat prolonged relaxation phase followed by a reversible contracture phase which is very pronounced. Certain predictable differences were obtained in concentrations between the two extremes.

The evidence presented would seem to indicate that muscles when undergoing the process of fatigue are qualitatively susceptible to differences in concentration of the medium with which they are surrounded. This implies that an intimate relation exists between the physical state of the muscular envelope (plasma-membrane) and the changing physiological conditions within.

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AMOEBOID MOVEMENT, TISSUE FORMATION AND CONSISTENCY OF PROTOPLASM

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Received for publication February 4, 1921

In a series of preceding papers we have attempted an analysis of the conditions which lead to the changes in the blood cells of Limulus after they have left the body of the animal. These changes concern the clot formation, the shape of the cells, the granules, amoeboid movement and the consistency of the cell (1). In this paper we shall analyze the effect of changes in osmotic pressure and constitution of medium, the effect of changes in temperature and of the character of the cellfibrin on the amoeboid movements in their dependence upon the consistency of the cell, and we shall discuss some wider bearings which our experiments may have on the theory of tissue formation and tissue movements in general.

We used the method described in a previous paper. We prepared experimental cellfibrin tissue and observed the migration of cells from this tissue in the hollow slide. In order to eliminate the effect of variable factors, such as variations in the size of the piece, in the amount of fluid added and others, we made a very large number of preparations.

It will not be necessary to review again the literature on amoeboid movement. This has been done repeatedly especially by Rhumbler. The most generally accepted view is that amoeboid movement depends on primary changes in surface tension in a liquid medium. This view has again been upheld quite recently by Tait (2).

Effect of osmotic pressure of medium on rate and amount of outgrowth. Of all the media used the outgrowth was found best in Limulus serum, at least in the beginning of the experiment. On the second day, or perhaps somewhat earlier, the growth may become equally good or even somewhat better in a m/2 NaCl solution. In solution of a higher osmotic pressure than m NaCl no outgrowth occurs. In m NaCl only a trace of growth is seen. In $\frac{3}{4}$ m NaCl there is a distinct outgrowth,

but it is usually considerably less than in solutions of $\frac{5}{8}$ m NaCl — m/2 NaCl. In these solutions generally the optimal growth is reached. In m/4 NaCl it is in the majority of cases less good than in m/2 NaCl, but occasionally it may be equally good; only in a very small minority of cases was it better. In m/6 and m/8 NaCl it is much less good than in m/2 NaCl.

In interpreting these results we must consider the probability that gradually substances are extracted from the tissue and added to the solution. This not only would tend to increase the osmotic pressure of the solution, and thus shift the optimum toward the originally hypotonic solutions, but in addition it might tend to add to the NaCl solution substances which render the constitution of the solutions more similar to the body fluid. In determining the effect of the medium on the tissue we have to take into consideration not only the width of the zone in which migrating amoebocytes are found, but also the density of the field (the number of outgrown cells) and the character of the cells, the preservation of the granules, the character of the pseudopodia and the intensity of the spreading-out process.

The influence of osmotic pressure on the character of pseudopods and amoeboid movements. The character of the pseudopods and amoeboid movement in the amoeboyctes of Limulus is not fixed but is variable and the variations depend upon a number of variable factors, such as character of cellfibrin tissue, temperature, difference in chemical composition of the surrounding medium and osmotic pressure.

We shall here discuss the typical effects caused by changes in osmotic pressure in NaCl solutions leaving the other factors approximately constant. This will be followed by a discussion of the other variable factors.

In m NaCl solutions the pseudopods are sharp, fine, threadlike or they are sharp tongue pseudopods. In $\frac{3}{4}$ m NaCl the cells are usually relatively small, contracted, granular. The granules appear small and are close together. The cells begin to spread out in contact with the glass, but the intensity of spreading out and the number of spread-out cells is restricted. NaCl tends gradually to lead to a solution of the granules.

Usually the pseudopods are here fine and long, threadlike; sharp pointed tongue pseudopods are also quite common. The thread pseudopods are very often multiple and very long, they may show a tree-like ramification so that structures not unlike glia cells are produced; or the appearance may be similar to bipolar or multipolar ganglia cells. At

podia in different media.

I. *a*, Circulating blood cells in the normal animal; they are flat plates. *b*, Rounded off cell after it has left the body. *c* and *d*, Effect of a sharp rise in temperature.

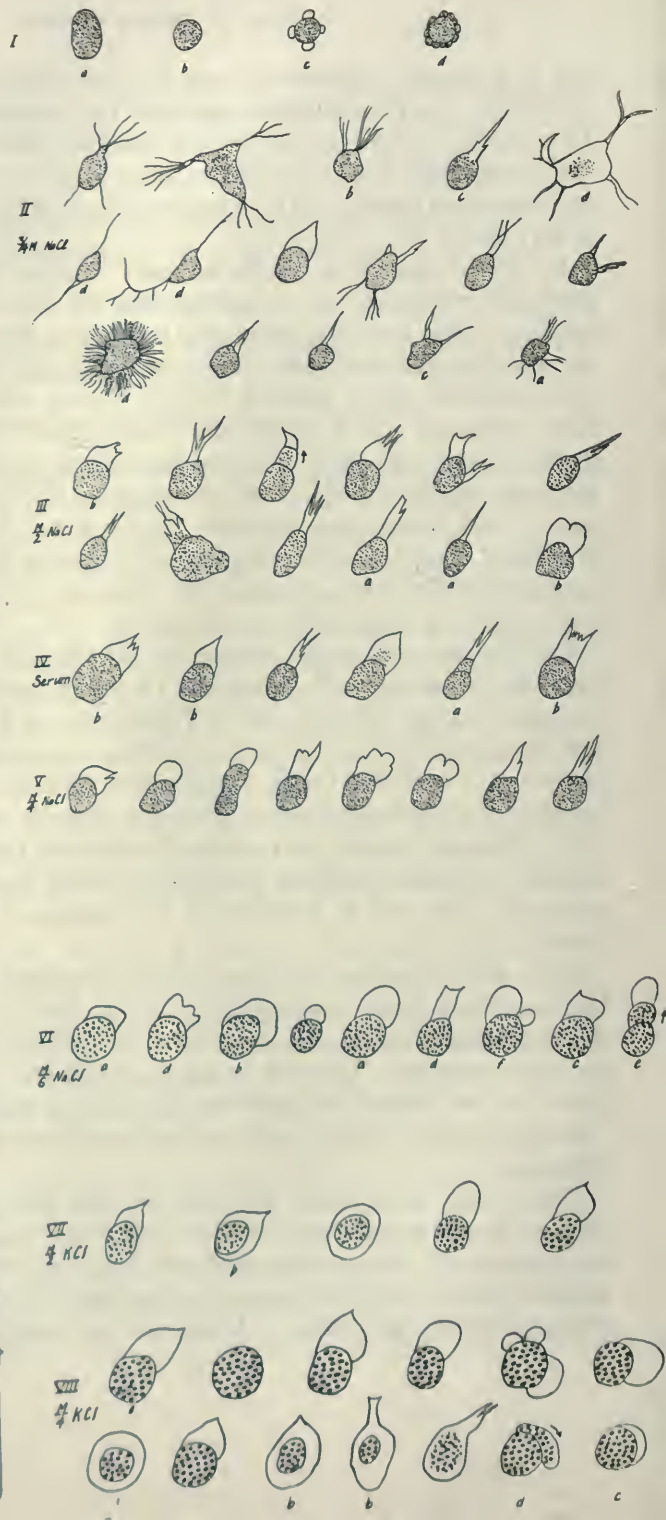
II. Hypertonic medium. *a* and *b*, cells with thread pseudopodia; *c*, sharp tongue pseudopodia; and *d*, cells resembling ganglia and glia cells.

III and IV. Isotonic medium. *a*, sharp tongue pseudopodia; *b*, broad tongue pseudopodia.

V. Slightly hypotonic medium. The broad tongue pseudopodia prevail.

VI. Markedly hypotonic medium. *a*, balloon pseudopodia; *b*, court pseudopodium; *c*, transitional pseudopodium; *d*, broad tongue pseudopodia; *e*, successive balloon pseudopodia with immigration of granuloplasm; *f*, multiple balloons.

VII and VIII. Action of potassium in isotonic and slightly hypotonic solution. *a*, Structures resembling ova with fertilization membranes; *b*, pseudopodial processes developing in such cells; *c*, cell with half-moon; *d*, cell with circus movement; *e*, transitional form.



the angles of triangular cells processes are attached which are very long, become finer in their distal parts and split dichotomously. In other cases we find very fine long dichotomous threads sitting on broader tongue pseudopods. In certain cases we may even find broad tongue pseudopodia, drops and small balloons in $\frac{3}{4}$ m NaCl solutions. This occurs especially if cellfibrin tissue is used which has a very low degree of resistance and if the tissue has been exposed to the temperature of the incubator. But even in these cases the drops and balloons are usually smaller than in non-hypertonic solutions and we are apt to find transitions to tongue pseudopodia. After having been kept for several days, even in the ice chest, many cells are flat, spread out, hyaline, and these cells usually show long branching pseudopods. These long threadlike pseudopods change even $\frac{3}{4}$ m NaCl solution, they may be partly drawn in and be sent out again at other places. On the whole the movement of the granuloplasm and of the hyaloplasm tends to be slow in this solution, and the granuloplasm is apt to move as a connected mass, although occasionally some granules may advance isolated into the pseudopod. We find here also successive formation of tongue pseudopodia. The granules move not only into the broader pseudopods, but occasionally also into fine threadlike pseudopods, the interior of which is less consistent than the periphery.

If we observe the cells emigrating from a piece of cellfibrin tissue in $\frac{3}{4}$ m NaCl solution, in which the temperature has been raised by placing the specimen soon after it has been made, under the microscope near an electric lamp, we may notice the appearance of very sharp pseudopods with which the cells emigrate from the tissue. In beginning however the pseudopods may not yet be quite so sharp as later, when the solution has had time to act on the cells and when perhaps the hypertonicity of the solution has been still further increased through substances extracted from the tissue.

In cells which have become more or less hyaline in the solution we may notice a framework of hyaline glistening, apparently relatively solid hyaloplasm from which protrude long fine pseudopods of a similar consistency, and which includes a vacuolar space in which the nucleus is situated. Some granules which are left in the cell move in contact with the hyaline framework or with fibers which are suspended in the vacuole, and it appears almost as if the granules were attached to the more solid structures. However, they become free and move in the vacuole, as soon as the fibers are dissolved. In $\frac{3}{4}$ m NaCl the amoebocytes show only a slight agglutination; the marked clump formation as we find it

especially in serum but also in some other solutions, is here usually absent.

In $\frac{5}{8}$ —m/2 NaCl solutions the cells are at first contracted granular. Gradually they spread out in contact with the glass and become hyaline; the cells which are not in contact with the glass remain contracted for a longer period and they keep their granules for a considerable time, but ultimately some of these cells may also become hyaline. The extending cells are at first still granular. Agglutination of cells into clumps is here usually more marked than in $\frac{3}{4}$ m NaCl, but less so than in blood serum. In m/2 NaCl the pseudopods are usually sharp, medium or broad tongue pseudopods with a prevalence of the sharp type. Some thread pseudopods may also occur. Under the same conditions, when drops and balloons appear in $\frac{3}{4}$ m NaCl solution, they may likewise appear in m/2 NaCl. This is especially the case whenever less resistant tissue is used and if the tissue had been kept at a higher temperature. But in m/2 NaCl these drop and balloon pseudopods are more frequent than in $\frac{3}{4}$ m NaCl and they may even occur spontaneously at room temperature in older specimens and especially in the most advanced cells which are more directly exposed to the salt solution. If we observe under the microscope the cells emigrating from the tissue in a freshly prepared specimen near a heat emitting lamp circus movement may occur, but it is very rare; it occurs under conditions which favor balloon formation.

Three-eighths m NaCl and m/4 NaCl: Only a few experiments were carried out with $\frac{3}{8}$ m NaCl, in the majority of cases m/4 NaCl was used. Here the same variety of pseudopodia and movements are found as in m/2 NaCl, namely, sharp, medium and broad tongue pseudopodia, drops, balloons and half-moons; but the relative frequency with which these different types are observed is different in m/4 and m/2 NaCl. Broad tongue pseudopodia, drops, balloons and half-moons are here relatively more frequent. The same factors which favor the formation of the latter in isotonic solutions have the same effect in m/4 NaCl; but they are more effective in the latter solution. These factors are length of time, during which the cells are exposed to the solution, higher temperature and the use of less resistant tissue. After exposure to the incubator temperature drops and balloons are apt to appear; they are more frequent in the peripheral, most advanced cells, than in the central cells. Circus movements may appear, but are rare.

M/6 and m/8 NaCl solutions: We can treat these two solutions jointly; their effect is essentially the same, although it is more pronounced in the latter solution.

Tongue pseudopodia as well as drops, balloon pseudopodia, half-moons and courts may be seen, but the balloons, drops, courts and half-moons are here relatively much more frequent than in the less hypotonic solutions. Balloons, courts and half-moons may be considered as the typical pseudopodia in these hypotonic solutions, but broad and even sharp tongue pseudopodia may also occur; they are the more frequent, the more resistant the tissue is, the less the time is which has elapsed since the specimen was exposed to the solution, the less the tissue was exposed to a higher temperature. We observe here occasionally *pari passu* movement of the granuloplasm into the half-moon; thus a partial extension of the cell may take place. In this condition the granuloplasm enters the pseudopods at once. The cell on the whole in this solution is rather large, the granules are well separated from each other and they are large. In consequence the cells are apt to appear as dark granular cells under the low power of the microscope. The cells are soft and the granuloplasm usually moves rapidly into the balloons. Successive balloons also occur, and the cell as a whole may show locomotion by these means.

If we observe a piece of tissue in these hypotonic solutions near a lamp at a higher temperature, we notice in the first hour and a half after the specimen has been made, an emigration of granular cells with large balloons, which are usually directed toward the outside and into which the granuloplasm moves. The large majority of the cells show these balloons in the first 90 minutes. Then the number of cells which emigrate with tongue pseudopodia becomes more frequent; and a number of cells may begin to show broad tongue pseudopodia or transitions between tongue pseudopodia and balloons in the solution. Under certain conditions sharp and broad tongue pseudopodia may even predominate in these solutions. It is possible that gradually substances are extracted from the tissue which increase the osmotic pressure of the solution and make it also otherwise more adequate for the blood cells.

A broad tongue pseudopod may explode into a balloon and a balloon may become transformed into an irregular tongue pseudopod. Thus transitional forms are produced. Slight circus movements are also occasionally observed. The same cell may alternately show broad tongue pseudopodia and balloons, and balloons may also be polarized, appear preferably at a certain pole of the cell. Balloons appear here much earlier than in solutions with a higher osmotic pressure and again they are more frequent in less resistant fibrin and at higher temperature.

If we lower the osmotic pressure of the medium still further by placing the tissue in distilled water, no outgrowth occurs. As I have shown previously blood cells do not under those conditions show those phenomena which we observe in hypotonic salt solutions, but the water changes the cell substance throughout in such a way that amoeboid movements are no longer possible. The cell becomes round, polygonal or leaflike and the majority of granules disappear; only exceptionally may we find a cell which shows a very broad or balloon pseudopod under those conditions. This is probably at places where through extraction of substances from the tissue the osmotic pressure of the fluid has been raised.

In blood serum of Limulus the typical pseudopodia are the various kinds of tongue pseudopodia; but here also some drops or even half-moons may form under certain conditions. The factors which favor the appearance of round pseudopods are the same as those mentioned previously: namely, spontaneous changes taking place in the cells, after a certain time—usually several days—has elapsed, the use of less resistant tissues and the use of higher temperature.

If we compare the behavior of the cells in Limulus serum and approximately isotonic NaCl solutions (m/2 and $\frac{5}{8}$ m), we find the following differences. In serum the tongue pseudopods are on the average broader than in NaCl solutions. In serum the cells tend to spread out with granules intact, while in NaCl solutions the cells are either more contracted, or if they do spread out especially in contact with the glass they lose part of their granules earlier than in serum. In serum the cells are agglutinated in small clumps to a greater extent than in NaCl solutions, where they are distributed more evenly.

Effect of various salt solutions on the amoebocytes. In our former investigations we studied the effect of various substances on the amoebocytes of Limulus. At that time we considered especially the cell consistency and the fate of the cell granules and only secondarily were we concerned with the character of pseudopods in these solutions. In our recent experiments we studied more particularly the effect of these solutions on the amoeboid movements. So far we gave special attention to the action of KCl, because we found previously that the various potassium salts seemed to have a softening influence on the protoplasm of the blood cells. We also noticed that the pseudopodia were less sharp in isotonic KCl than in NaCl solutions.

Effect of m/2 KCl on the amoeboid activity. If we place a piece of tissue in a m/2 KCl solution on a cover glass, we find only a very lim-

ited outgrowth of cells. The majority of the cells are round and granular; some of the cells become hyaline. Pseudopodial activity is limited; we may find sharp and broad tongue pseudopods but also somewhat swollen cells with courts, half-moons and drop pseudopodia, and others presenting structures resembling eggs with fertilization membranes; furthermore pseudopods transitional between tongue and balloon or drop pseudopods. Here also the character of the tissue is of some effect, and also the temperature effects are noticeable, but even in tissue kept in the ice chest some results of softening, as the formation of courts can be observed. If the softening effect in the cytoplasm is sufficiently marked, Brownian movement of the granules may be seen in the cells.

If we use $\frac{3}{4}$ m KCl solution, which is somewhat hypertonic, we observe round granular or hyaline fixed cells in contrast to a $\frac{3}{4}$ m NaCl solution, where the cells show very sharp fine pseudopods. The most characteristic effects of KCl however are obtained in m/4 solution which is somewhat hypotonic. Here the cells are usually round, rather large; the granules are larger and distinct and the cell appears dark, granular. The outgrowth in this solution is usually slight, similar to that in m/8 NaCl. If we observe under the microscope the outgrowth of cells from the tissue near the lamp soon after the specimen has been made, we find many cells with large balloons which are directed outward, away from the tissue. The granuloplasm moves into these balloons.

Other cells move out with irregular balloons which form transitions to broad tongue pseudopods. Here also the granules move into the balloons rather rapidly. If we observe the cells somewhat later, after they have been in this solution for some time we find in addition to the balloons cells with half-moons, courts; other cells resemble eggs with fertilization membranes. There are occasionally some cells with tongue pseudopodia, or transitional forms between balloon and tongue pseudopodio, but usually they are absent. A court pseudopodium may also send out an irregular process.

The balloons usually form on different sides of the cells successively and granules enter the balloons. The balloons in a m/4 KCl solution are very large, larger than in any other solution so far investigated.

The cell as a whole may move if the granuloplasm enters the balloon. If we observe at room temperature tissue in m/4 KCl solution soon after it has been taken from the ice chest, we usually find balloons and courts in the cells; these balloons change their position, are drawn in at one place and sent out at another. Granules enter these balloons and courts; but we usually do not find any circus movements which we

described in our previous paper. These circus movements consist in the rapid formation of a balloon; this balloon begins to move in a circle around a part of the cell circumference and simultaneously the granuloplasm pours as a whole into the balloon and follows the circus movement of the latter. Thus the whole cell, the hyaloplasm as well as the granuloplasm of the cell has become irregular in outline. Through the subsequent retraction of the balloon which presses upon the granuloplasm, both granuloplasm and hyaloplasm may again become rounded off and the cell take the appearance of a ball.

Such circus movements are, as stated, usually absent in cells kept at a lower temperature, but they appear and often in many cells simultaneously, as soon as the temperature of the specimen is raised, by placing the microscope near a lamp. Usually a cell performs those circus movements for some time; then the movement stops and the cell is at rest for a short time, when the movement begins again.

We thus see a distinct effect of temperature on the circus movements of balloons. In this regard they behave like other pseudopodial movements and in accordance with the effect of temperature on the rapidity of outgrowth. Also in m/4 KCl solution the outgrowth of the cellfibrin tissue is greater at incubator than at room temperature, or in the ice chest. Often the circus movements are only partial, the peripheral part of the granuloplasm carrying out these movements, while the central part remains stationary.

The first effect of warming the cells seems to be an increased Brownian movement of the granules within the cell; this is followed by the circus movements. In some cases the softening of the granuloplasm which is a prerequisite of the typical circus movement occurs without the formation of balloons. In such cases the granuloplasm may show a very active streaming within the cell without any subsequent outflowing of the granuloplasm, and without any circus movements following. In this case we have a condition analogous to protoplasmic movements within the cell such as is found in plant cells, unaccompanied by pseudopodial movement.

We have stated previously that in some cases even in a m/4 KCl solution some cells show the formation of tongue pseudopods. If we follow the fate of such cells, we notice that they usually continue to send out tongue pseudopodia, while the cells with balloons and circus movements continue to produce balloons, and circus movements. Exceptionally it may be seen that in a cell which presents a tongue pseudopod, a balloon with subsequent circus movements may develop.

These circus movements may continue in a specimen for several days. As we have stated they are only active at a higher temperature. However, if the temperature exceeds a certain optimum, the movements cease; thus while at the temperature of the incubator of about 34° circus movements may be seen at first, they cease here much earlier than in cells kept at a somewhat lower temperature.

The characteristic feature of the action of m/4 KCl is its effect on the granuloplasm. It shares the production of the balloons, half-moons, courts with hypotonic solutions of NaCl; but even in this case the action of m/4 KCl is much more marked than in a NaCl solution of equal osmotic pressure. In a m/4 KCl solution the balloons are much more frequent and often larger than in a m/4 NaCl solution. In addition a m/4 KCl solution softens the granuloplasm in such a way that it flows readily into the balloon, or in some cases moves actively within the cell and shows protoplasmic streaming. The mass of granules are apparently held together by a mass of denser protoplasm which at the periphery is perhaps so dense that it assumes the character of a second membrane. Under the influence of the m/4 KCl this membrane or mass of dense protoplasm is softened or partly dissolved and the granuloplasm flows very readily. Sometimes the softening or solution of the membrane may be localized and affect only a limited area of the membrane; then we see an opening in the periphery of the granuloplasm through which it streams into the balloon. At other times the softening is general.

We see then the action of m/4 KCl is essentially a softening, dissolving one. Not only is here the granuloplasm softened, but also the layer of exoplasm which forms the outer layer of the pseudopod. There is evidence which points to the conclusion that in a balloon this layer is softer than in a sharp tongue pseudopod, such as is found in isotonic or hypertonic solutions.

There are two components in this action of m/4 KCl. First, the softening effect of KCl, which is already indicated in isotonic solutions and secondly a weakly hypotonic effect which greatly increases the effect of the KCl but which in itself would be unable to produce the effects observed in a m/4 KCl solution.[1]

[1] We have found the effect of M/4 KCl identical in two different preparations of KCl, one a Kahlbaum preparation and the other a purified KCl preparation obtained from the laboratory of Doctor Denis at the Massachusetts General Hospital. It is therefore not probable that the effect of the solution was due to accidental admixtures.

In accordance with our previous observations we find that in solution of NH_4Cl the majority of the cells remain more or less unchanged as round or oval or slightly irregular granular cells; they appear almost as fixed cells; but in m/2 NH_4Cl some tongue pseudopodia and some drops and balloons may develop. In m/4 NH_4Cl the number of drops and balloons and of half-moons is greater; granules may pour into them through openings in the periphery of the granuloplasm; occasionally also a tongue pseudopod or a transitional pseudopod may be seen. There is very little active movement taking place in this solution, although there may be a layer of round granular cells on the upper as well as the lower surface of the fluid which surrounds the tissue. It is probable that at least many of these cells were passively detached from the piece, although some active movement may occur. Here also the cell movement is more considerable at higher than at lower temperature.

If we observe under the microscope near a lamp the tissue in a m/4 NH_4Cl solution soon after the specimen has been made, we notice some drops and balloous into which granules move. After one-half hour some larger balloons may be formed, some of which are directed toward the piece. Occasionally cells come out of the piece with tongue pseudopods, but outside of the tissue balloons are formed.

With $CaCl_2$ very few experiments were made. As in our previous experiments we found that m/2–m/4 $CaCl_2$ solutions produce a hyaline condition of the cells, which latter may be extended or contracted. They have glistening refractile protoplasm, which often includes a large vacuole; a few sharp pseudopods may form. The outgrowth in this solution is very slight.

In the literature I could find only some isolated observations, indicating that under certain conditions a change in the shape of a cell can be produced. Thus Zacharias observed that in a 5 per cent solution of Na phosphate certain spermatozoa and even an intestinal cell formed pseudopods and cilia (3). Verworn observed that if the water in which amoeba lived and moved was made faintly alkaline in the course of 15 to 20 minutes the character of the pseudopods changed to that characteristic of amoeba radiosa (4).

Effect of mixtures of salts on the outgrowth of cellfibrin tissue. If we remove the protein constituents of Limulus serum by boiling and filtering, a marked deterioration takes place; the outgrowth in the remaining fluid is very much diminished in the large majority of experiments. However, in certain cases the result is better, approaching even that in unchanged serum, although not quite reaching this level. As far as our

experiments indicate, this variation is due mainly to variations in the character of the cellfibrin used. If this is very resistant, the outgrowth may be relatively good. The same applies to sterile sea water. Here also the outgrowth is much less good than in serum and it may be lacking altogether, if the cellfibrin is not very resistant. The difference between serum and sea water under those conditions is very marked; but even with resistant cellfibrin the difference, while not so marked, is quite distinct.

Sea water in Woods Hole is alkaline in reaction. If we use instead a neutral mixture of salts, of similar composition to sea water (Van't Hoff solution) the results may become somewhat better, but so far the effect has not been so good as in serum even under the most favorable circumstances; the outgrowth has been less and the migrating cells have become hyaline at an earlier date than in serum. We find here again the same dependence upon the character of the cellfibrin used.

From this we may conclude that the proteins of the serum exert a protective influence upon the blood cells and at the same time, as we shall show elsewhere, that they are specifically adapted to the blood cells.

The effect of temperature on the rate of outgrowth. In a number of experiments we compared the rapidity of outgrowth in specimens kept in the ice chest (8°–10°), at room temperature (on the average 19°–24°) and in the incubator (about 34°). We found that the rate of outgrowth was more rapid at higher temperature; it was most rapid at the temperature of the incubator, least rapid in the ice chest. The difference between the rate of outgrowth at the different temperatures varies considerably in the individual experiments. Usually it was less than should have been expected according to the van't Hoff rule. This variability is due to variations in the size of the pieces, amount of fluid used and similar factors; but in addition there is another factor at work which tends to counteract the beneficial effects of the higher temperature. A rise in temperature accelerates not only those processes which lead to pseudopodial activity, but simultaneously it accelerates other processes in the cells which are unfavorable to pseudopodial activity, namely, a spreading out of the cells in contact with the glass, a process which is accelerated by a rise in temperature up to a certain limit. A further rise favors contraction (the exact temperature at which this change takes place remains still to be determined). Furthermore at a higher temperature there takes place a more rapid solution of granules and other changes which modify pseudopodial activity unfavorably.

In addition it is to be assumed that the larger number of cells which leave the cellfibrin tissue at the higher temperature, and spread out near the glass, present an obstacle to the subsequent outgrowth of other cells.

All these factors tend to counteract the favorable effects of a rise in temperature and thus the results are somewhat variable and usually less marked than should be expected according to the van't Hoff law.

The effect of temperature on the rate of outgrowth was noticeable not only in serum, but also in various salt solutions, even in m/2 KCl and m/4 KCl. the outgrowth being more considerable at the higher temperature.

Effect of temperature on the character of amoeboid movement: a. If we keep pieces taken from the same tissue surrounded by the same medium, in the ice chest, at room temperature and in the incubator (at about 33°–34°), we notice that the influence of the medium on the character of the pseudopods is noticeable in all the specimens irrespective of the temperature; the hypotonic effects of m/4–m/8 NaCl, the hypertonic effects of $\frac{3}{4}$ m NaCl, the influence of isotonic solutions and the characteristic effects of m/4 KCl solutions were distinct at low as well as high temperatures. There was however noticeable in many, although not in all experiments, some effects of a rise in temperature. In the first place the movements of hyaloplasm as well as of granuloplasm were more active at a higher temperature. The Brownian movement was more noticeable. We mentioned already that the circus movements of the cells suspended in m/4 KCl began only at a higher temperature. There was furthermore a tendency for the pseudopods to become broader, more rounded off at a higher temperature and to be sharper, thinner at a lower temperature. Thus the balloons, drops, courts and intermediate pseudopods seemed relatively more numerous at incubator than at room temperature, and still more so at ice chest temperature. This relative preponderance of the broader pseudopods at a higher temperature could be found in all kinds of solutions. This difference however was not observed in every case. Whether this effect was noticeable depended probably to some extent on the character of the tissue used. It will be necessary to carry out further experiments in order to make possible more definite statements as to the conditions under which this influence of temperature becomes noticeable.

b. There is however quite definite another effect of temperature which can be obtained regularly.

If we expose a specimen in a hollow slide to a higher temperature by placing it near an electric lamp in such a way that a thermometer being held in direct contact with the specimen reaches a temperature of 39° to 43° in about 3 to 12 minutes, and if we then remove the specimen from the direct effect of the lamp and place it for examination under the microscope we observe definite changes which have in common, *a*, the contraction of many blood cells and *b*, the production of droplets and small balloons.

If we heat a specimen in the manner indicated, we frequently observe under the microscope at first tongue pseudopodia forming in round granular contracted cells. Within 2 to 5 minutes this is followed by the formation of droplets or small balloons on the surface of the contracted cells. This continues for 10 or 15 minutes or even for a longer period. Isolated granules or the connected granuloplasm enter these droplets, and inasmuch as these droplets are often numerous, the filling of these droplets by granules may cause the appearance of mulberry forms. The unevenness of the cell outlines thus produced gradually disappears again and in the end, after 10 to 20 minutes, new tongue pseudopodia appear and subsequently the cell begins gradually once more to expand. We find thus a distinct cycle of changes as the result of the heating, first formation of tongue pseudopodia, then appearance of single or multiple drops and small balloons, and in the end again tongue pseudopodia.

However, in many cases this cycle is not complete. Sometimes we find at once the formation of drop pseudopodia to which are perhaps admixed a few tongue pseudopodia. These may persist throughout, or they may be followed in the latter stage by a mixture of drops and tongue pseudopodia in which the latter gradually become more prevalent, or may be the only ones visible in the last period. It is not quite clear what these differences depend upon. It is probable that tissues which in the unheated condition tend to the formation of balloon and drop pseudopodia and courts show after heating a similar tendency toward the formation of pure drops.

Insufficient heating is another cause which may lead to the formation of tongue instead of drop pseudopodia. Heating up to 36° to 39° within a period of 5 to 7 minutes often is not sufficient to cause the production of drops, but calls forth the formation of tongue pseudopods.

Again we find here an interchange between tongue and drop or balloon pseudopods. Thus drop or balloon pseudopodia may develop

spines and thus become tongue pseudopods. Or a tongue pseudopod may in its entirety or partially explode into a drop or balloon. The same cell may at one time show tongue pseudopods, later drops and then again tongue pseudopods; or a cell may show simultaneously at one place drops, at another place a tongue pseudopod. At a certain stage of the cycle there may appear successive balloons, which form at the same pole of the cell and into which the granuloplasm moves, thus insuring a locomotion of the cell as a whole.

These changes under the influence of heating occur in solutions differing as much as m/6 NaCl on the one hand and $\frac{3}{4}$ m NaCl on the other. They appear also in serum and in Van't Hoff solution. They develop quite readily in $\frac{3}{4}$ m NaCl solution; here either the complete cycle may appear or merely multiple drops. In all these media the drops are relatively small, but as far as can be judged without accurate measurements they are smaller in $\frac{3}{4}$ m NaCl than in isotonic or hypotonic solutions.

In m/4 KCl heating does not call forth the formation of typical small droplets, but the ordinary balloons may continue to be produced after heating. In heated cells at first no circus movements appear, but later they may again take place. Also in other solutions slight circus movements may appear in cells which have been heated previously. In m/4 KCl solution heating stimulates in some cases the formation of tongue pseudopods. In m/2 NH_4Cl a reaction is obtained with greater difficulty. In a number of cases heating does not call forth the production of tongue pseudopods or drops. In other cases some drops or tongue pseudopodia may be produced. In m/4 NH_4Cl likewise heating remains either without effect or it causes the formation of droplets and balloons. We see then that a certain reactive state of the protoplasm and a certain consistency is required in order to obtain these responses.

It is possible to obtain with the same specimen the same cycle repeatedly. After one cycle has been finished the specimen may be heated again and a new response is called forth. We obtained this reaction as often as 5 or 6 times in succession with the same specimen.

If we put the specimen later into the ice chest for several hours, the cells begin to expand again in their usual manner, and through heating again the typical reaction may be obtained. However, the return to the lower temperature does not necessarily mean a complete return to the old condition of the protoplasm. The tissue has to some extent been definitely changed through the previous heating and in the ice chest certain cells continue to produce balloons, drops or courts, although

tongue pseudopodia may also appear. A moderate heating of such tissue under the microscope near the lamp may again temporarily stimulate the production of tongue pseudopodia.

Differences in the amoeboid activity of different cellfibrin tissues. As we have mentioned previously, the cells migrating from different kinds of cellfibrin of Limulus behave in certain cases differently, while in other cases the cellfibrins are equal. Usually fresh, 1- to 2-day old tissue is better than 5-day old tissue, and the latter is superior to 10-day old fibrin. But this is not always the case; other factors besides age play a part in determining the behavior of the tissue. Thus cellfibrin which has partly retracted after standing for 5 days or longer in the ice chest was found inferior even if parts of the tissue were used which had not yet retracted. In such cases the tendency to retraction is present even in such parts of the tissue in which the change is not yet visible. The elastic tension under which the tissue is held has increased. Usually such tissue is not equal to fresh cellfibrin.

The state of health of the Limulus from which the experimental tissue was obtained is of special significance. If an anemic individual served for this purpose, the cellfibrin is less active and resistant, and younger tissue from an anemic, weak individual may be inferior to the somewhat older tissue from a healthy Limulus. On the other hand I did not find a noteworthy difference between tissue prepared from blood which gave a deeply blue color after standing and other tissues obtained from somewhat paler blood.

The differences between various tissues may be as follows: 1, The more resistant healthier and younger tissue shows usually a better outgrowth. *2*, In such tissue the cells have usually a greater tendency to preserve the granules than in inferior tissue, where in some cases the cells become hyaline more rapidly. *3*, The character of the pseudopods differs in both cases. In inferior tissues there is on the whole a greater tendency to the formation of balloon and drop pseudopods, while in very healthy young tissues the tendency is greater toward the formation of tongue pseudopodia. Of course the character of the media in which the tissue is placed determines how far that tendency is realized; but in hypertonic ($\frac{3}{4}$ m NaCl) as well as in hypotonic solutions (m/6 NaCl) this tendency finds expression.

The degree of variation caused by differences in the experimental tissue varies greatly in different cases and may also vary in different solutions. While in certain experiments the difference between different tissues may be relatively slight in favorable media such as healthy

Limulus serum unheated or heated to 56°, 60° or 70° or m/2 NaCl, it is very much more pronounced if we use more unfavorable media, as, e.g., Limulus serum heated to 100°, very old Limulus serum, heterologous serum, seawater or even Van't Hoff solution. In such cases there may be a considerable outgrowth if good tissues are employed, while the outgrowth is very slight or lacking altogether if the inferior tissue is used.

The factors which determine the direction of movement of cells from the cellfibrin tissue into the surrounding medium. As in the case of other tissues, the constituent cells of which are motile, we can also in the case of the experimental Limulus tissue recognize two factors which determine the direction of outgrowth, namely, *a*, The stereotropic response which induces tissue cells to move in contact with a solid base (this reaction was first described by us in 1898 and later by Harrison and others); and *b*, the tendency to centrifugal growth.

The former we explained on the basis of our observations in amoebocytes as due to changes in consistency in the surface layer of the cells which make them sticky. We pointed out the relationship between this phenomenon and other phenomena, like phagocytosis, the formation of foreign body giant cells, and the giving off of substances accelerating the coagulation of the blood on the part of blood cells. In all these cases the contact with a solid body, strange to the life of the normal cell, leads to the response, which apparently everywhere consists in a diminution in the consistency of the cell surface. In some cases this leads to an agglutination of cells, such as we find in the formation of experimental cellfibrin tissue; in other cases the diminution in consistency of the cell surface proceeds so far that two cells which at first stick together become united into one. This may take place in the formation of foreign body giant cells and in the formation of other kinds of syncytia.

The tendency to centrifugal growth leads to a radial outgrowth which we notice in the growth of various tissue elements and which is especially noticeable if tissues grow in the test tube. In previous papers we considered the possibility that differences in electric potential between the side of injury and the normal side of the tissue might direct the outward growth of the cells. However, some preliminary experiments which we carried out in the summer of 1919 and of 1920 (the latter were done in association with Mr. Samuel E. Pond) and in which we exposed small pieces of cellfibrin tissue in a medium of Limulus serum to the action of a constant galvanic current, so far failed to show any influence of the current on the migration of the cells.

In addition we carried out several experiments in which we exposed in a dark room the experimental tissue kept as usual in hollow slides to the beam of an electric lamp. In each experiment a number of slides were placed in the field of the light rays, control slides were kept in the dark. The intensity of the light which was used varied approximately between 2500 and 1000 meter candles.[2]

The time of exposure varied approximately between 4 and 7 hours in different experiments. The tissues were suspended in serum, in m/2 NaCl and in m/4 NaCl. There was no noticeable difference between the outgrowth on the side directed toward or away from the light, neither was there a marked difference between these specimens and control specimens kept in the dark. Neither the direction nor the intensity of the outgrowth was consistently influenced by the light.

In other experiments we found that if we place two pieces of tissue near each other, either in a dish or on a coverglass in a hollow slide, the tissues do not exert a repellent effect upon each other; the cells coming from each piece grow toward each other. The same result we obtained in experiments in which we placed two pieces of mammalian tissue in the test tube; both pieces were surrounded by coagulum of blood plasma. The cells coming from the two pieces were not repelled by each other. If we observe the direction in which the pseudopods are sent out we notice that in the cells which emigrate from the piece of tissue the pseudopods are directed outward; this applies also to the balloon and drop pseudopods which form in certain media. In cells which have moved away from the tissue the majority of the pseudopods are likewise usually directed outward, but others point in the opposite direction, namely, toward the piece, or they are sent out in a lateral direction. Cells may move toward each other and meet and stick together. Subsequently the agglutinating cells send out pseudopods in such a way that the cells become again separated from each other.

On the basis of our observations and experiments the centrifugal outgrowth may be interpreted by assuming that contact with a cell leads to a resting condition at the place of contact and that the strange environment surrounding the cells elsewhere produces changes in consistency of the surface of the cell and leads to the production of pseudopods. This would insure a tendency of the cells to move away from each other. After the cells have once left the piece of tissue their centrifugal growth is to some extent a chance phenomenon. They have a

[2] I wish to express my thanks to Dr. Selig Hecht who put his apparatus at my disposal for these experiments.

much better chance to move without finding an obstacle in migrating in the direction away from the tissue; in moving toward the tissue they meet much more frequently other cells to which they stick and from which they become separated only by the sending out of pseudopods in an outward direction. Thus according to the law of chance the tissue would spread out more and more in a centrifugal direction.

It is furthermore probable that there exists a tendency for a cell to persevere in the sending out of pseudopodia at a certain pole. We often notice that tongue as well as balloon pseudopodia are sent out consecutively at the same pole. At other times, however, the direction changes; this is especially frequent in the case of the rapidly forming pseudopods, the balloons and drops. But it occurs also in the case of tongue pseudopods. Pseudopods can be sent out at any part of the cell surface; but we noticed that if a drop pseudopod had developed at one pole and this drop had been subsequently filled out by granules, the opposite pole was favored for the formation of tongue pseudopods, while the place where the drop had formed remained at rest at least for some time afterward.

If we consider all these factors we come to the conclusion that chance plays an important part in the centrifugal growth of the tissue.

The Limulus clot considered as a tissue. Further light is thrown on the character of the pseudopodia and of amoeboid movement of the amoebocytes by a consideration of the blood clot of Limulus.

In my earlier papers on the blood of arthropods I pointed out that the blood clot of Limulus represents a tissue which is free from fibrin. I showed that under certain conditions structures can be obtained which resemble connective tissue (in isotonic solutions, as a result of mechanical factors), under other conditions structures are produced which resemble epithelial tissues (in hypotonic, slightly alkaline solutions). Last summer I observed that by the use of slightly hypertonic solutions structures can be obtained under certain conditions which closely resemble nerve and glia tissue. While of course no identity exists between such tissues and the experimental structures, yet I believe that these analogies are of significance and indicate conditions under which the normal tissues develop. They point to the conclusion that differences in the consistency of the various cells which constitute these tissues play a part and that these differences in consistency are to some extent analogous to those which lead to the experimental production of these structures in vitro. I believe furthermore that in the normal tissue formation changes in the consistency of the surface layer of the cells

which lead to agglutination play a similar rôle to that found in experimental tissue and that the sending out of amoeboid processes and movements of granules into the pseudopods are of significance in both cases. Also the effect of pull and pressure is similar in the case of the normal tissues and of Limulus clot.

In 1919 we showed that the essential processes of wound healing can be imitated in tissue which has been prepared experimentally in such a way that an even layer of agglutinated cells is produced.

There is however a special reason why the resemblance of the Limulus clot to a tissue should be discussed in this connection. The typical Limulus tissue is a solid structure, which resembles in this respect fibrin and connective tissue. It resembles also fibrin and connective tissue in that it retracts spontaneously and especially if cuts are made into the tissue. Now this elasticity is dependent upon a change in the physical character of the cells after they have begun to spread out or after a part of the cell protoplasm has been drawn out or flown out from ruptured cells. These structures acquire elasticity and their outer layer becomes gelatinous. This applies especially to the pseudopods which are sent out by the cells. We have thus an additional proof of the change in consistency of the cell protoplasm during amoeboid movement; but while the changes in consistency during amoeboid movement are reversible, in tissue formation we have to deal with further-going changes which are no longer completely reversible. However, some cells which are widely spread out, and connected with other cells by means of long pseudopodia and form thus a part of a tissue can in certain cases again be made to contract by exposure to a higher temperature.

We see therefore that the cell protoplasm after it has spread out acquires certain new physical properties which cause it to contract. It is conceivable that an analogous change in physical properties is also at the base of the contractive phase in amoeboid movement. The latter would represent an acute process, while the tissue retraction would represent the same phenomenon as a slow, non-reversible process.

In previous papers I showed that in Limulus blood no conversion of fibrinogen into fibrin takes place; a real coagulation does not occur here, but merely an agglutination of blood cells (5). This coagulum is therefore comparable to a tissue. Last summer I found additional facts which confirm this conclusion. We could show that in all those arthropods in which a true coagulation takes place, I did not succeed in preparing an experimental cellfibrin tissue by the methods employed

in the case of Limulus blood. The fibrin which surrounds the clumps of agglutinated blood cells prevents the outgrowth of the amoebocytes. The clot of lobster, bluecrab, spidercrab, rockcrab blood cannot be used as experimental tissue.

That our interpretation of this phenomenon is correct follows also from the fact that if we surround a piece of Limulus cell fibrin with a layer of uncoagulated plasma of lobster, bluecrab or rockcrab blood, the surrounding clot prevents the outgrowth of the cells. While the serum obtained from the corresponding clot permits an outgrowth, the amoebocytes do evidently not develop a sufficient kinetic energy to overcome the mechanical obstacles presented by the fibrin.

We may therefore conclude that in the blood clot of Limulus such an envelope of true fibrin is absent.

On the character of amoeboid movement and tissue formation. In 1901 we observed that the sending out of pseudopods in amoebocytes depended upon a change in consistency of the protoplasm of the cells. During the process of formation the cytoplasm was soft and very soon afterward the outer part of it became solid (6). Our subsequent observations confirmed the solid character of the pseudopods (7) and especially Jennings noticed the solid character of pseudopods also in amoebae (8).

We interpreted therefore in our earlier paper amoeboid movement as due to changes in consistency of the protoplasm comparable from a mechanical point of view to the transformation of fibrinogen into fibrin, but a reversible process. The recent studies of Pauly and especially Jacques Loeb on proteins make understandable such reversible changes which take place whenever the neutral protein changes either through combination with acid or alkali or even with a neutral salt into a protein salt. Under those conditions the protein takes up H_2O and changes in viscosity take place. On this basis we explained previously the changes in stickiness and in shape which occur in the blood cells as soon as they leave the body of the animal. In agreement with this interpretation are the marked changes which we observed in the physical properties of the blood cells under various conditions. These changes in consistency and elasticity were tested by us directly by exerting pressure and traction upon the blood cells and by rolling them up between glass slides (9).

Secondarily added to these changes in consistency are changes in surface tension which take place whenever the changes in consistency lead to a sufficient liquefaction. These we noted in certain hypotonic solutions, but particularly in slightly hypotonic KCl solutions. The

balloons and drop pseudopodia, the courts and half-moons show very clearly the influence of surface tension.

In this paper we have shown that we can experimentally change the consistency of the protoplasm and that the character of the pseudopodia corresponds to the consistency. This is a further proof in favor of the conclusion that the formation of pseudopods depends upon changes in consistency in the protoplasm. These changes in consistency not only modified the character of the pseudopods but the whole cell and a very viscid consistency of the protoplasm is associated with pseudopods whose character agrees with such a viscid condition. The same correspondence we find between a soft, liquid protoplasm and the character of the pseudopods. We can experimentally grade the consistency and accordingly the pseudopodia. The size and distance of the granules, their fixedness or their ability to carry out Brownian movements, the character of the movement of the granuloplasm, whether it moves slowly, connectedly as a whole or rapidly and as individual granules, the size of the cell, indicate the character of the protoplasm.

As we have stated the number of amoebocytes which show the typical response to the changed environment varies in different cases. Not all the cells present the typical pseudopodia in hypotonic or hypertonic solutions. Certain cells are evidently more resistant to the osmotic changes in the environment. In addition, as we have stated previously, it is probable that especially in hypotonic solutions gradually an increase in osmotic pressure occurs through solution of certain cell constituents. These changes in the medium in which the cells are placed not only modify the character of the cytoplasm, but also the rigidity of the pseudopodial exoplasm. While normally it resembles solid gelatine, it is somewhat softer in the balloon pseudopods. Here it adapts itself to the internal pressure of the fluid and can readily be pushed in through pressure from the outside. It appears therefore that the degree of solidification of the external protoplasm may also vary in intensity. We saw that all possible transitions exist between the typical tongue pseudopodia and the balloon and drop pseudopods; furthermore one kind can be transformed into the other kind and the same cell can produce in succession first one, then the other kind. Moreover we saw that the cell as a whole, particularly the granuloplasm, behaves toward these drop and balloon as it does toward the other pseudopods—it moves into them.

As we have stated in our previous papers the majority of the granules in the amoebocytes of Limulus are situated near the surface of the cell.

Preceding amoeboid movement the consistency of the exoplasm becomes more fluid. This change is strictly localized and leads to the formation of a sharp spine or sharp tongue; this is often followed by a widening of the area of softening and thus a somewhat broader tongue is formed at the base of the spine and lifts the former up. This is the most frequent mode of origin of the typical tongue pseudopodia. In other cases, however, a pseudopod starts as a broader, rounded-off pseudopod and secondarily develops on top a sharp ridge or spine.

While this process of partial liquefaction is taking place and the softened hyaloplasm flows out assuming a shape in accordance with the degree of viscosity of the substance, and in certain cases in accordance with the amount of and the degree of fluidity of outflowing endoplasm, the granules underneath the softened area seem to be held together by a more viscous material. Gradually a pull is exerted on this granuloplasm and it also moves into the pseudopod. This is perhaps accompanied by a process of partial liquefaction in this material surrounding the granules. Sometimes it appears as if a sharp opening were produced through liquefaction in a shell separating the granuloplasm from the pseudopod and this opening permitted now the granules to pour into the pseudopods. This is observed especially under conditions under which the consistency of the whole cell has been diminished. It is therefore very probable that changes in consistency occur not only in the exoplasm, but also in the granuloplasm. Especially marked is this softening of the granuloplasm under conditions which lead to the formation of circus movements.

The formation of the pseudopods depends then upon processes of liquefaction and upon a separation of the more liquid and the more solid parts of the cell, and in this respect the formation of pseudopods, the formation of droplets in cytolysis and the formation of a fertilization membrane in the mature ovum are related phenomena. We have produced experimentally in the amoebocytes analogous processes. While the mode of formation of the fertilization membrane and of the corresponding structures in the amoebocytes is not identical, the principle underlying the formation of both structures is the same. The typical formation of pseudopods represents a change of medium intensity while the formation of the structures resembling eggs with fertilization membranes on the one hand and long ramified threads on the other represents extreme processes in a continuous series.

As we have seen, it is possible to change the character of the amoeboid movement and of the pseudopods, not only through a change in

the medium in which the blood cells are suspended, but also through changes in the temperature of the medium. We saw that a rise in temperature above that of the room favors a contraction of the blood cells and that even cells which in contact with the glass had spread out can again contract. If the rise in temperature is still greater, the cycle of changes takes place which we have described above; prominent among these changes is the temporary formation of multiple drop pseudopodia and mulberry cells.

In addition it is at least probable that a higher temperature in general favors the broadening of pseudopods. The most probable interpretation of these phenomena is that it is due to a greater liquefaction of the protoplasm produced by the increase in temperature. If the liquefaction (as indicated by the Brownian movement of the formerly fixed granules, which is sometimes noticeable) is sufficiently marked, the cell rounds off, loses its stickiness, becomes partially or entirely detached from the slide and sends out drop instead of tongue pseudopodia. This change is to some extent reversible; the cell protoplasm becomes again more consistent and sticky and spreads out, if returned to the ice chest. However, as we have seen, a permanent change in consistency of the protoplasm has been produced through this heating. Changes which take place spontaneously but slowly have been thus accelerated.

With this interpretation would agree the observations of Greeley who found that a lowering of the temperature causes a solidification of the protoplasm, while raising the temperature has the opposite effect and favors the taking up of water (11). K. Gruber observed in amoeba proteins that exposing the organisms to a temperature of 30° for several hours causes the formation of a peripheral hyaline layer which Gruber interprets as due to liquefaction; returned to cool water, hyaline drop-like processes formed, which gave the amoeba the appearance of a mulberry; this is subsequently followed by a resumption of the normal movements (12). There is apparently some similarity between these processes and those observed by us in blood cells of Limulus after heating. The rise in temperature sets in motion a cycle of changes associated with an increase in the fluidity of the cell.

It is very probable that the droplike formations which A. Golubew (13) and R. Klemensiewicz (14) produced in the leucocytes of amphibian blood through the application of interrupted electric currents are of a similar nature. Strong stimuli of various kinds would then produce similar changes in consistency of the protoplasm and similar reactions.

We conclude then in accordance with our previous interpretation that changes in consistency of the protoplasm are the primary factors in the amoeboid movement of amoebocytes and of the leucocytes in general, and that changes in surface tension may follow secondarily. That it holds good also in the case of amoebae is made probable through the observations of Jennings (15), Dellinger (16), E. Schultz (17), Kite (18), Mast (19), Chambers (20) and Hyman (21). Even Rhumbler who following Berthold, Quincke and Verworn maintains that in general amoebae are liquid and that the amoeboid movement is primarily due to changes in surface tension in a liquid medium, has admitted that in certain cases changes in consistency of the protoplasm play a part (22). We believe the evidence points to the conclusion that in general changes in consistency are the primary factor in amoeboid movement, that surface tension changes follow secondarily and that only if the liquefaction has proceeded sufficiently far may perhaps surface tension changes represent the primary factor.

Our observations on the conditions which modify amoeboid movements have a bearing also on the analysis of tissue formation as we attempted it on the basis of our experiments. The stickiness which develops in the isolated cells as a result of mechanical and chemical stimuli, and which leads to agglutination and is thus at the base of the tissue formation, cannot be caused primarily by changes in surface tension in a liquid substance, but must be due to changes in consistency similar in character to those taking place in gelatine when it becomes sticky. These changes are probably dependent upon changes in the relation between proteid molecules and the surrounding water. Closely related to these changes are those upon which depends the stereotropism of tissue cells, phagocytosis and the formation of foreign body giant cells, as we have shown on previous occasions.

The stereotropic reaction consists in a spreading out of the cell and the changes associated with this process lead to a creation of potential energy. The spread-out cells and cell fibers are held under tension and retract when cut. In the course of their formation elasticity has developed. This is probably also the cause of the retractibility and tension which we find in various natural tissues. These are likewise due to these changes in consistency of the protoplasm.

SUMMARY

1. The amount of outgrowth from the amoebocyte tissue depends upon the osmotic pressure of the medium. The optimal point is at a concentration of about m/2 NaCl. From there a decrease takes place toward both sides, the decrease increasing with diminishing and increasing osmotic pressure.

2. The factors on which the behavior of amoebocytes emigrating from (experimental) cellfibrin tissue (rapidity of movement, degree of the stereotropic spreading out, preservation of granules, character of pseudopodia and amoeboid movement, consistency of the protoplasm) depends are the following: *a*, character and age of the tissue used; *b*, osmotic pressure and character of substances in the medium surrounding the tissue; *c*, temperature; *d*, time during which the tissue is exposed to the medium. It is possible to modify experimentally the majority of these factors and thus to modify the character of the pseudopods and of the amoeboid movement.

3. Through changes in osmotic pressure of the surrounding medium it is possible to change the consistency of the cell and concomitantly the character of the amoeboid movements.

4. K, NH_4 and Ca have typical effects on the cells and their amoeboid movements. KCl in a slightly hypotonic solution leads to a marked softening of the whole cell, including the granuloplasm and thus it is possible to produce circus movements of the cell and occasionally intracellular plasmastreaming. These movements do not usually occur at a temperature of 10°; they are accelerated by a moderate rise in temperature (to about 25°–28°).

5. Mixtures of salts, similar to those found in the blood have so far not given results as good as Limulus serum. They are especially inferior to serum, if cellfibrin is used which is less resistant.

6. A rise in temperature increases the rate of outgrowth from the cellfibrin tissue. This outgrowth however is usually less marked than should be expected according to the Van't Hoff rule. This is probably due to the fact that a rise in temperature favors at the same time destructive changes in the cells.

A rise in temperature also increases the protoplasmic movements within the cell. Under certain conditions it leads to a broadening and rounding off of the pseudopods. If the rise in temperature is sufficiently sharp a cycle of changes can be produced, the most typical part of

which is the formation of multiple drop pseudopodia and of mulberry forms. Although these changes are to some extent reversible, heating causes a permanent alteration of the protoplasm.

7. The cells derived from different cellfibrin tissues differ in their behavior. Older tissues and tissue obtained from anemic animals manifest their inferiority especially in less favorable media.

8. The migration of cells from tissues is determined by two factors: *a*, the stereotropic reaction, and *b*, the tendency toward centrifugal growth. The former depends upon changes in consistency of the protoplasm induced by changes in environment and especially by contact with solid bodies. As we have pointed out previously, the same reaction plays a part in phagocytosis, in the formation of foreign body giant cells (where the foreign body may produce still further-going changes in the cell consistency) and in the giving off of substances accelerating the coagulation of the blood. The tendency toward centrifugal growth can best be explained by assuming that contact with a cell leads to a resting condition at the place of contact, while the change in environment elsewhere leads to movement. To some extent the centrifugal growth would thus be a chance of phenomenon. So far we have not found either a galvanotropic or a phototropic reaction in the amoebocytes.

9. Additional proof is given that the clot of Limulus blood is not true fibrin but consists merely of agglutinated cells. It is shown that this clot can be considered as the type of a tissue and that the factors underlying the formation of this clot are similar to those active in tissue formation; prominent among those factors are changes in the consistency of the protoplasm. Gradations in the consistency of the protoplasm lead to the production of various structures which are analogous to various normal tissues.

10. It is probable that amoeboid movement in blood and tissue cells as well as in protozoa, the agglutinative processes underlying the formation of normal and experimental tissue are due to primary changes in consistency to which may be added secondarily changes in surface tension. It is furthermore probable that the formation of pseudopods and the processes leading to the formation of a fertilization membrane in the mature ovum are related phenomena.

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A STUDY OF THE DISTRIBUTION OF IODINE BETWEEN CELLS AND COLLOID IN THE THYROID GLAND

III. The Effect of Stimulation of the Vago-Sympathetic Nerve on the Distribution and Concentration of Iodine in the Dog's Thyroid Gland

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Received for publication February 5, 1921

For many years it has been held that the thyroid gland is supplied with true secretory nerves. In support of this assertion there is considerable anatomical evidence and some physiological evidence. Of late the nerves which anatomists have traced into the thyroid gland and have considered to be possibly secretory in function have been declared to be branches of the cervical sympathetic nerve. And recent physiological work has tended to confirm this view. Only twelve years ago Wiener (1) published the report of experiments from which he concluded that extirpation of the inferior cervical ganglion produces a marked atrophy of the thyroid gland on the side of the extirpation. Wiener maintained that no comparable effect on the lobe of the thyroid gland on the side of the operation could be produced by vagotomy or by removal of the superior cervical ganglion. More recently Rahe et al. (2) announced that they were able to produce a quite marked diminution in the iodine concentration of the lobe of the thyroid gland on one side by stimulating the thyroid nerves in several different ways. They stimulated the nerves of the superior thyroid artery, the intact vago-sympathetic nerve as well as the vago-sympathetic nerve near the level of the superior cervical ganglion after ligating the nerve low in the neck and cutting the nerve central to the point of stimulation. They found that the most marked loss was brought about by the stimulation of the intact vago-sympathetic nerve.

Watts (3) undertook to find out whether or not the results obtained by Rahe, Rogers, Fawcett and Beebe might be due to vasomotor

changes in the gland on the stimulated side. Watts likewise found that he could reduce the iodine content of the right or left lobe of the thyroid gland of the dog by stimulating the "cervical sympathetic isolated from the vagus sheath" and the "nerve filaments accompanying the superior thyroid vessels." However Watts maintained that he could cause some diminution in the iodine content simply by periodically reducing the blood flow through the gland by "occluding the main thyroid artery" the nerves of which had been dissected away. Hence he concluded that all of the effects of stimulation on the iodine content can be accounted for by the coincident vasomotor changes which he showed to be present.

Positive evidence of the secretory effect of sympathetic stimulation has been reported by Cannon and his co-workers (4) in several communications. Working with cats they sutured the phrenic nerve with the cervical sympathetic nerve and observed following the operation increased basal metabolism, respiratory hippus and falling hair which they interpreted as the results of hypersecretion of the thyroid gland caused by the periodic bombardment of the gland by impulses carried from the respiratory center to the gland's secretory nerves. Cannon and Cattell (5) adduced additional evidence as to the rôle of the sympathetic nerves in experiments dealing with the electrical condition of the gland. Following the stimulation of the upper thoracic sympathetic nerves or the injection of epinephrin they were able to show a definite action current in the thyroid gland after a latent period of five to seven seconds. Recently Cannon and Smith (6) maintained that gentle massage of the thyroid gland or stimulation of the cervical sympathetic nerve increases the rate of the denervated heart. The denervated heart is said not to be affected when the cervical sympathetic nerve is stimulated after removal of the thyroid gland. Moreover Levy (7) observed that the pressor effect of epinephrin after a variable latent period is increased by the stimulation of the cervical sympathetic nerve. He declared that stimulation of the cervical sympathetic nerve has no such effect after thyroidectomy.

However the conclusions based on the experiments mentioned above have not been universally accepted. Burget (8) was unable to alter the thyroid gland noticeably either by uniting the phrenic and cervical sympathetic nerves or by removing a section of the cervical sympathetic nerve. Marine, Rogoff and Stewart (9) sutured together the phrenic and cervical sympathetic nerves in several cats. They demonstrated a functional union between the phrenic and cervical sympathetic nerves

but observed no exophthalmos, tachycardia or respiratory hippus in their animals. There was no apparent difference either grossly or histologically in the lobes of the thyroid gland on the operated and the non-operated sides. Troell (10) reported that he was unable to produce either exophthalmos or respiratory hippus by suturing the proximal end of the phrenic nerve to the cervical sympathetic nerve. Employing cocaine as a sensitizer for sympathetic nerve endings, Mills (11) did not observe, following the repeated injection of cocaine, any alteration in the amount or nature of the thyroid secretion as judged by what is known of the gland's histology. Finally Rogoff (12) records one experiment in which he drew blood from a vein of the left lobe of the thyroid gland and at the same time stimulated the cervical sympathetic nerve on that side in the hope of increasing the secretory activity of the stimulated lobe. From the right lobe he also collected blood by way of a vein. While drawing the blood he massaged the right lobe to some extent but did not stimulate the right cervical sympathetic nerve. He found that specimens of dried blood from each lobe were potent when fed to tadpoles. But he could detect iodine chemically only in the blood from the non-stimulated lobe. Moreover the non-stimulated lobe had a lower iodine content than the stimulated lobe.

In connection with some studies on the distribution of iodine in cells and colloid in the thyroid gland I attempted to alter acutely the total iodine content of the gland by stimulating the vago-sympathetic nerve of the dog. Some inconsistencies in the results in the early part of the work forced me to investigate the matter more carefully and to repeat the work of Rahe et al. (2) and of Watts (3).

Methods. Dogs were used in all of the experiments. All except those whose numbers are above that of no. 78 were given daily feedings of iodine over a period of one to eleven days. The daily feeding consisted of a capsule containing two drops of tincture of iodine in starch. In the animals fed iodine the stimulation of the vago-sympathetic nerve was undertaken from two to ten days after the last feeding of iodine. Throughout the experiments the animals were *lightly* anesthetized with ether. Platinum wire electrodes were applied to opposite sides of the carefully isolated vago-sympathetic nerve and shielded from all surrounding tissues by glass. In all of the experiments except those recorded in table 5, a tetanizing current from three to six times as strong as that sufficient to bring about a pupillary dilatation and apparent protrusion of the bulbus oculi was employed. The regulation of the strength of the current was made possible by a rheostat inserted in the

circuit. Again in all of the experiments except those to be found in table 5 the stimulating current throughout the period of stimulation was made for about 0.8 second at intervals of 1.6 seconds by means of a clock and ratchet device. The strength of the current used and the rate at which the current was made in the experiments of table 5 are described below.

TABLE 1

Variations in the iodine content of neighboring specimens of the same lobe of the thyroid gland

ANIMAL NUMBER	NUMBER OF FEEDINGS OF IODINE	LOBE OF THYROID GLAND	WEIGHT OF SAMPLE OF DRIED GLAND ANALYZED	IODINE IN DRIED GLAND ANALYZED	DIFFERENCES IN THE CONCENTRATION OF IODINE IN NEIGHBORING SPECIMENS OF THE SAME LOBE
			gram	*per cent*	*per cent*
68	11	Left	0.1192	0.331	0.093
			0.1378	0.424	
		Right	0.1525	0.418	0.037
			0.1668	0.455	
74	6	Left	0.6931	0.366	0.005
			0.6420	0.371	0.034
			0.6846	0.405	0.039
		Right	0.4549	0.370	0.006
			0.4931	0.364	0.019
			0.5533	0.383	0.013
88	0	Left	0.4454	0.121	0.011
			0.5298	0.132	
		Right	0.7857	0.119	0.010
			0.4528	0.129	
105	0	Left	0.7241	0.158	0.018
			0.7138	0.176	
		Right	0.4489	0.163	0.015
			0.4516	0.148	

After a number of experiments had been performed it was found that there is considerable variation in the iodine content of adjoining pieces of the *same* gland. In table 1, for example, are given a few analyses of neighboring specimens of the same gland. The differences in neighboring portions of the glands are somewhat greater in iodine-fed animals.

TABLE 2

Quantitative determination of iodine in whole gland and in cells free from colloid material of thyroid glands of dogs whose isolated vago-sympathetic nerve had previously been stimulated for approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF WHOLE GLAND USED	IODINE IN WHOLE GLAND	WEIGHT OF CELL MASS USED	IODINE IN CELL MASS	RATIO OF PER CENT OF IODINE IN CELLS TO PER CENT OF IODINE IN WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN WHOLE GLAND OF STIMULATED LOBE
			gram	*per cent*	*gram*	*per cent*		*per cent*
42	Left	2 hrs. 15 min.	L 0.1792	0.531	0.2533	0.371	0.699	−0.029
			R 0.1693	0.560	0.1360	0.303	0.541	
45	Left	3 hrs. 0 min.	L 0.4602	0.148	0.5117	0.011	0.074	+0.046
			R 0.5336	0.102	0.4750	0.014	0.137	
46	Left	3 hrs. 30 min.	L 0.4982	0.098	0.3445	0.014	0.143	−0.006
			R 0.5252	0.104	0.2284	0.017	0.163	
54	Right	2 hrs. 30 min.	L 0.3964	0.177	0.2713	0.036	0.203	−0.030
			R 0.4241	0.147	0.3883	0.019	0.129	
55	Right	2 hrs. 40 min.	L 0.6880	0.232	0.2700	0.031	0.134	−0.046
			R 0.5509	0.186	0.2280	0.046	0.247	
56	Right	2 hrs. 10 min.	L 0.4536	0.121	0.3683	0.016	0.132	−0.012
			R 0.4795	0.109	0.3059	0.014	0.128	
62	Left	3 hrs. 5 min.	L 0.0836	0.494	0.0480	0.078	0.158	−0.029
			R 0.0558	0.523	0.0612	0.082	0.157	
64	Right	3 hrs. 50 min.	L 0.0892	0.110	0.1019	0.008	0.073	+0.017
			R 0.0692	0.127	0.1190	0.010	0.079	
65	Right	3 hrs. 0 min.	L 0.1179	0.436	0.1086	0.037	0.085	+0.030
			R 0.1419	0.466	0.1064	0.052	0.112	
66	Right	3 hrs. 0 min.	L 0.0709	0.485	0.0733	0.041	0.085	+0.008
			R 0.0593	0.493	0.0783	0.038	0.077	
67	Left	3 hrs. 0 min.	L 0.1373	0.464	0.1842	0.041	0.088	+0.013
			R 0.1237	0.451	0.1613	0.040	0.089	
68	Left	2 hrs. 45 min.	L 0.2570	0.377	0.1703	0.031	0.082	−0.060
			R 0.3193	0.437	0.1379	0.028	0.064	
69	Left	3 hrs. 0 min.	L 0.5360	0.605	0.2627	0.083	0.137	+0.036
			R 0.3957	0.569	0.1476	0.092	0.162	
71	Right	3 hrs. 0 min.	L 0.3651	0.346	0.1718	0.026	0.075	−0.041
			R 0.2990	0.305	0.1310	0.029	0.095	

Hence it was thought desirable to analyze not single blocks or samples of dried powdered mixtures of whole gland but to analyze the *whole* gland in each case. In experiments in which this last mentioned technique was employed the whole gland was carefully cleaned of connective tissue and blood vessels, and thoroughly dried first over an electric hot plate and then in an electric oven. After two to three hours' drying in the electric oven the gland was broken into several pieces whose weight was about 0.5 gram each and whose number therefore depended on the size of the gland. The iodine determinations were made according to the method earlier described by Kendall (13). By analysis of powdered thyroid of known iodine content the accuracy of the method (to about 0.008 mgm. of iodine) and the purity of the reagents used were frequently examined and found to be satisfactory.

The strength of the current employed in all of the experiments recorded in table 2 was three times that sufficent to cause dilatation of the pupil and apparent protrusion of the bulbus oculi. Both the stimulated and the non-stimulated vago-sympathetic nerves were cut in two places: at a point in the neighborhood of the eighteenth tracheal ring and also at a level a little above that of the hyoid bone. The reason for cutting the non-stimulated nerve in such a manner was to eliminate the possible effect of tonic secretory impulses on the non-stimulated lobe. The vago-sympathetic nerve was stimulated a little above the point at which it was cut low in the neck. In the above experiments the ratio of the percentage of iodine in cells to the percentage of iodine in whole gland was determined by a method previously described (14). From the data given in table 2 it may be seen that stimulation of the vago-sympathetic nerve under the conditions described is without appreciable effect on either the ratio value or the concentration of iodine in the whole gland.

When it was found that there was no consistent diminution in the concentration of iodine in the stimulated lobe only the stimulated vago-sympathetic nerve was sectioned in the manner described above. In experiments 76, 79 and 80 a strength of current six times that necessary to cause ocular changes characteristic of sympathetic stimulation was used; in all of the other experiments to be found in table 3 the current was of the same strength as that used in the experiments recorded in table 2. From the standpoint of the iodine concentration in whole gland the results given in tables 2 and 3 are very similar. Stimulation apparently has no effect on the concentration of iodine in the stimulated lobe.

By stimulating the intact vago-sympathetic nerve Rahe et al. (2) declare that they were able to produce the most marked diminution in the iodine content of a given lobe of the thyroid gland. In the three experiments of table 4 of my series, the intact vago-sympathetic nerve was

TABLE 3

The concentration of iodine in the lobes of the thyroid gland of the dog after the stimulation of the isolated vago-sympathetic nerve on one side for a period of approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
			gram	*per cent*	*per cent*
72	Right	2 hrs. 50 min.	L 1.2883	0.186	+0.003
			R 1.4258	0.189	
74	Right	3 hrs. 10 min.	L 2.0197	0.381	−0.008
			R 1.5013	0.373	
75*	Left	3 hrs. 10 min.	L 1.4975	0.334	−0.026
			R 1.8796	0.360	
76	Right	3 hrs. 10 min.	L 0.4653	0.770	+0.051
			R 0.3509	0.821	
77	Right	3 hrs. 30 min.	L 0.7055	0.471	+0.037
			R 0.7914	0.508	
79	Right	3 hrs. 15 min.	L 0.2421	0.474	+0.024
			R 0.1995	0.498	
80	Left	4 hrs. 0 min.	L 0.6342	0.465	−0.012
			R 0.4850	0.477	
82	Left	3 hrs. 10 min.	L 0.8483	0.203	−0.003
			R 0.7516	0.206	
83	Left	3 hrs. 15 min.	L 1.2435	0.105	+0.002
			R 0.9163	0.103	
84	Left	3 hrs. 10 min.	L 0.7582	0.129	−0.016
			R 0.6022	0.145	

* Entire right lobe not analyzed.

stimulated in the neck at about the level of the fifteenth tracheal ring. The current was of the same strength as that employed in the experiments of table 2; each time the current was made there ensued a respiratory arrest and the ocular changes typical of sympathetic stimulation. Again there was no consistent change in the concentration of iodine in the stimulated lobe.

TABLE 4

The concentration of iodine in the lobes of the thyroid gland of the dog after the stimulation of the intact vago-sympathetic nerve on one side for a period of approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
			gram	*per cent*	*per cent*
78	Left	3 hrs. 40 min.	L 0.4628	0.528	+0.027
			R 0.3706	0.501	
86	Right	3 hrs. 20 min.	L 0.7039	0.138	−0.013
			R 0.6919	0.125	
88	Left	3 hrs. 15 min.	L 0.9752	0.127	+0.004
			R 1.2385	0.123	

Effect of vasomotor activity on the concentration of iodine in the thyroid gland. It will be recalled that Watts (3) concluded from his experiments that vascular changes will account for the diminution in the concentration of iodine which he brought about by stimulation of the cervical sympathetic nerve. The experiments of table 5 were undertaken to find out whether or not a slightly different type of stimulus sent into the nerve at an interval more nearly like that employed by Watts had an effect comparable to that found in the experiments previously performed. It was also thought desirable to determine whether or not the characteristic vascular changes were present throughout the experiments.

The technique except for certain features of the stimulation was the same as that used in all of the preceding experiments. Usually the stimulated vago-sympathetic nerve was ligated and cut at about the level of the eighteenth to twentieth tracheal ring; near the ganglion nodosum only the vagus nerve was cut. In all of the experiments the sympathetic chain was intact above the eighteenth tracheal ring. The

TABLE 5

The concentration of iodine in the lobes of the thryoid gland of the dog after the stimulation of the vago-sympathetic nerve, the sympathetic portion of which was left intact above the point of stimulation

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	PRESENCE (+) OR ABSENCE (—) OF VASOMOTOR ACTIVITY AT END OF EXPERIMENT		WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (—) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
					grams	*per cent*	*per cent*
101	Right	3 hrs. 10 min.	—	L R	1.8213 1.5890	0.177 0.125	−0.052
102	Left	3 hrs. 0 min.	+	L R	0.1397 0.1727	0.141 0.120	+0.021
103	Right	3 hrs. 0 min.	+	L R	0.6335 0.5749	0.277 0.274	−0.003
104	Right	3 hrs. 0 min.	—	L R	0.1891 0.1844	0.190 0.164	−0.026
105	Right	3 hrs. 25 min.	+	L R	1.4379 0.9005	0.167 0.156	−0.011
106	Left	3 hrs. 25 min.	+	L R	0.6161 0.6624	0.043 0.041	+0.002
107	Right	3 hrs. 0 min.	—	L R	3.1065 2.9642	0.103 0.109	+0.006
108	Left	3 hrs. 35 min.	+	L R	0.3462 0.2754	0.021 0.027	−0.006
109	Right	3 hrs. 25 min.	+	L R	1.0407 1.0116	0.014 0.016	+0.002
111	Left	3 hrs. 50 min.	+	L R	0.2875 0.2638	0.076 0.075	+0.001
113	Right	3 hrs. 30 min.	—	L R	1.5649 2.6585	0.034 0.033	−0.001
114	Right	3 hrs. 30 min.	+	L R	0.3210 0.3690	0.143 0.132	−0.011
115	Left	3 hrs. 45 min.	+	L R	1.0381 0.8196	0.088 0.090	−0.002
116	Left	3 hrs. 30 min.	—	L R	0.6650 0.6753	0.016 0.017	−0.001
117	Left	4 hrs. 20 min.	+	L R	1.1050 1.1701	0.140 0.134	+0.006
118	Right	5 hrs. 0 min.	—	L R	0.6568 0.5036	0.456 0.438	−0.018
119	Right	3 hrs. 10 min.		L R	0.6866 0.5695	0.168 0.173	+0.005

vago-sympathetic nerve was stimulated just above the point at which it was cut near the eighteenth tracheal ring. It was easily possible to vary the strength of the electrical stimulus so that a current of such a strength was employed as just to bring about the ocular changes characteristically associated with stimulation of the cervical sympathetic nerve. Mendenhall (15) has emphasized the markedly toxic effects of ether on the sympathetic nervous system. In a preparation like that last described the sensitivity of the cervical sympathetic nerve to the depressant action of ether could easily be demonstrated. A tetanizing current of rather low frequency from a Stoelting inductorium was made for 5.5 seconds at intervals of 11.8 seconds throughout the period of stimulation. At the end of most of these experiments a vein of the stimulated lobe, in most cases without the ligation of its companion veins, was cannulated and the effect on blood flow of stimulation of the gland under the *same* conditions as those employed in the experiment was observed. In ten out of sixteen experiments no difficulty was encountered in demonstrating a vasoconstriction in the gland on stimulating the vago-sympathetic nerve with an electric current of the same strength and delivered at the same rate as that used in the previous stimulation period. The threshold of excitability for the vasoconstrictor nerves of the thyroid gland appears to be considerably lower than that for the submaxillary gland as reported by Gruber (16). The relatively low threshold of excitability of the vasoconstrictor nerves of the thyroid to epinephrin stimulation has been observed by Gunning (17).

From table 5 it may be seen that stimulation of the vago-sympathetic nerve with the sympathetic trunk intact above the eighteenth tracheal ring has no appreciable effect on the concentration of iodine in the stimulated lobe. In a number of experiments it was unequivocally demonstrated that vasoconstrictor fibers to the gland were being stimulated; yet such stimulation did not alter detectably the iodine content of the lobe subjected to stimulation.

Effect of stimulation of the vago-sympathetic nerve on the concentration of water in the thyroid gland. In table 6 may be found data relative to the effect of stimulation of the vago-sympathetic nerve on the concentration of water in the thyroid gland. I was unable to find that stimulation had any effect on the concentration of water in the stimulated lobe even in experiments in which vasomotor changes were definitely proved to result from stimulation.

TABLE 6

The concentration of water in the lobes of the thyroid gland of the dog after the stimulation of the vago-sympathetic nerve for a period of from three to four hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	CONCENTRATION OF WATER	APPARENT GAIN (+) OR LOSS (—) IN CONCENTRATION OF WATER IN STIMULATED LOBE
			per cent	*per cent*
77	Right	3 hrs. 30 min.	L 69.25 R 69.64	+0.39
79	Right	3 hrs. 15 min.	L 73.40 R 74.26	+0.86
82	Left	3 hrs. 10 min.	L 74.06 R 74.66	−0.60
83	Left	3 hrs. 15 min.	L 77.57 R 78.02	−0.45
84	Left	3 hrs. 10 min.	L 74.38 R 74.24	+0.14
88	Left	3 hrs. 15 min.	L 73.65 R 72.81	+0.84
108*	Left	3 hrs. 35 min.	L 80.14 R 80.71	−0.57
109*	Right	3 hrs. 25 min.	L 78.54 R 80.64	+2.10
110*	Right	3 hrs. 45 min.	L 82.69 R 82.13	−0.56
111*	Left	3 hrs. 50 min.	L 78.03 R 77.33	+0.70
113	Right	3 hrs. 30 min.	L 75.98 R 73.73	−2.25
115*	Left	3 hrs. 45 min.	L 75.76 R 75.63	+0.13
116	Left	3 hrs. 30 min.	L 79.92 R 78.34	+1.58
117*	Left	4 hrs. 20 min.	L 76.53 R 75.65	+0.88
119	Right	3 hrs. 10 min.	L 75.90 R 76.81	+0.91

* Vasomotor effect of the stimulation demonstrated at the end of the experiment.

DISCUSSION

If we consider the data described above we find that following the stimulation of a given lobe of the thyroid gland there was no consistent change in the concentration of iodine in that lobe compared with the

TABLE 7

A comparison of the effect of the different methods of stimulation of the vago-sympathetic nerve on the concentration of iodine in the thyroid gland of the dog

SERIES	NON-SIGNIFICANT	APPARENT LOSS IN CONCENTRATION OF IODINE			APPARENT GAIN IN CONCENTRATION OF IODINE			REMARKS
		Number	Maximum	Average	Number	Maximum	Average	
			per cent	*per cent*		*per cent*	*per cent*	
I	2	7	0.060	0.035	5	0.046	0.028	Stimulated vago-sympathetic nerve cut above hyoid bone and low in the neck. Non-stimulated vago-sympathetic nerve cut similarly in most cases
II	4	3	0.026	0.018	3	0.051	0.037	Only stimulated vago-sympathetic nerve cut as in series I
III	1	1	0.013	0.013	1	0.027	0.027	Intact vago-sympathetic nerve stimulated at about level of fifteenth tracheal ring
IV	11	5	0.052	0.024	1	0.021	0.021	Vago-sympathetic nerve stimulated at about level of eighteenth tracheal ring. Sympathetic nerve entirely intact above point of stimulation
V	7	2	0.011	0.011	1	0.021	0.021	Animals of series IV in which vasomotor effect of stimulation could be shown plainly at end of experiment

nonstimulated lobe. Following stimulation there was an apparent diminution in the concentration of iodine in 24 or 54.5 per cent of the stimulated glands, and an apparent gain in 20 or 45.5 per cent of glands stimulated similarly. Watts (3) reported that the average difference

in the percentage of iodine in the lobes of dried thyroid gland of the dog is 0.015 per cent in this vicinity. If we consider as non-significant all differences in the percentage of iodine in dried thyroid of 0.010 per cent or less we see that stimulation, while causing an apparent diminution in the concentration of iodine in 16 or 36.4 per cent of the glands, and an apparent increase in the concentration of iodine in 10 or 22.7 per cent of the glands, had no effect on the iodine concentration of 18 or 40.9 per cent of the stimulated glands. The greater part of the experimental data presented may be briefly summarized in table 7.

The differences which I found above appear to be due to normal variations in the concentration of iodine in the two lobes of the dog's thyroid gland. The average percentage difference in the concentration of iodine in the two lobes of the dog's thyroid gland depends on a number of factors such as the type of gland, the time of year and the feeding of iodine. Hence I am forced to conclude that if stimulation of the vago-sympathetic nerve in the dog has any effect on the concentration of iodine in the thyroid gland, that effect is considerably less than the normal variation in the iodine content of the stimulated and control lobes. The presence of normal variations relatively so much greater than the variations which may follow stimulation renders valueless the application of more refined methods of iodine determination in the study of the effect of stimulation of the cervical sympathetic nerve on the concentration of iodine in the thyroid gland.

SUMMARY

1. Periodic stimulation of the isolated vago-sympathetic nerve by an induced current of a moderate to strong intensity over a period of from three to three and a half hours does not appreciably alter the distribution ratio of iodine between cells and colloid.

2. The findings of Rahe, Rogers, Fawcett and Beebe (2) and of Watts (3) that stimulation of the cervical sympathetic nerve for a comparable period of time reduces the concentration of iodine in the stimulated lobe were not confirmed. Watts' assertion that such stimulation reduces the water content of the stimulated lobe was not confirmed.

3. Conclusions as to the direct secretory control which the cervical sympathetic nerves exercise on the thyroid gland are based in no small measure on the alleged effect of stimulation of the cervical sympathetic nerve on the iodine content of the gland. Conclusions having such a basis apparently are untenable.

It is a pleasure to acknowledge the many suggestions and constant aid given me by Dr. A. L. Tatum during the progress of this work.

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EXTENSIBILITY OF MUSCLE: THE EFFECT OF STRETCHING UPON THE DEVELOPMENT OF FATIGUE IN A MUSCLE

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Received for publication February 5, 1921

The extensibility of muscle was first systematically investigated by Weber (1) in 1846. It was he who found that a muscle stretched by the addition of equal increments of weight does not follow Hooke's law. Its curve of extensibility is not a straight line but a hyperbola. Weber's findings have been confirmed beyond question. There are, however, some other organic substances, non-living, which do not follow Hooke's law when extended by weights (2). Therefore, is the muscle which is stretched by a weight purely passive, or, does it put forth an active effort to support the weight; that is, does the stretching of a living muscle involve an increase in its metabolism, an energy transformation, the doing of work?

Guerrini (3) considers the specific form of the curve of extensibility of muscle as affording evidence of a vital response in the muscle. He speaks of the later more gradual part of the curve as dependent upon the biological factor, arguing from the lessening or total disappearance of this part of the curve, the straightening out of the curve in other words, when the vitality of the muscle is lessened or destroyed. He cites particularly poisoning of the muscle by various means and fatty degeneration of the muscle as affecting the curve in this manner. Many observers are agreed that the extensibility of muscle is increased, the curve more nearly approaches a straight line, during contraction and with the development of fatigue.

Brodie (4) found that muscles in situ with intact nerve and blood supply were more resistant to extending forces, the same weight caused less lengthening, than isolated muscles or muscles in situ whose nerve supply had been cut.

Rood (5) in 1861 claimed to have demonstrated that a sudden stretching may serve as a stimulus and cause the muscle to contract. Fick (6), on the other hand, emphatically denied such a possibility. We have ourselves observed the phenomenon to take place in some of our experiments. If simply an incision is made over the gastrocnemius tendon in a decerebrated frog and the tendon freed and cut without dissection of the muscle, or interference with its nerve and blood supply, the application of a small extending force, 10 or 20 grams, is not infrequently followed immediately by a contraction of the muscle. We have seen the tone of the muscle so increased that its actual length was less than before the weight was applied.

Montgomery (7) appears to have been the first to conceive the possibility that the stretching of a muscle may be accompanied by chemical as well as physical changes within the muscle. He did not, however, venture to reveal the nature of these chemical changes.

Meyerstein and Thiry (8), Schmulewitsch (9), Westermann (10), Blix (11), Danilewsky (12) and Roy (13) all found that there is a rise of temperature in a muscle when it is stretched. Danilewsky, it is true, attempted to explain the temperature change as due to internal friction and Westermann and Roy observed qualitatively similar elevation of temperature in dead muscle and other tissues removed at autopsy; but the other authors quoted believed that there was an actual increased production of heat coincident with the extension.

Hill (14) also has recently recorded the production of heat when a muscle is extended by a weight. He gives to this heat production a twofold explanation: Based upon a mathematical formula the temperature should rise because the coefficient of thermal expansion for muscle is negative; there is a wasting of work with liberation of heat in overcoming viscous forces when the muscle is stretched at a finite rate.

Du Bois Reymond (15) and v. Korányi and Vas (16) reported a change in the electrical condition of muscle, a negative variation, when it is "regularly" stretched.

Wundt (17) and Nasse (18) found in their experiments that the muscle which has been stretched fatigues more quickly upon subsequent stimulation than one which has not been stretched. On the other hand, the possibility of a fatiguing effect of mere stretching has been denied by Kronecker (19), Harless (20) and Leber (21). It should be pointed out that the last two authors employed small weights and allowed them to act for short periods only.

Taking up the question at this point we have carried out a series of twenty-five experiments with a view to determining the possible extent to which the stretching of a muscle may influence the development of fatigue.

Both gastrocnemius muscles were removed from a pithed frog as speedily as possible with especial care to avoid traction on either muscle. Each was fastened in the femur clamp of a separate moist chamber of the Harvard type and fine wires were run from the base of the moist chamber to the muscle for its direct stimulation. A pin-hook with attached thread was passed through each tendo Achillis. One was left free; the other was fastened to a muscle lever and made to support a weight of 100 grams for varying lengths of time. Subsequently each muscle was stimulated with an induced current to the point of complete exhaustion. In two instances, experiments 4 and 21, a tetanizing current was employed; in all of the other experiments the stimuli were single break shocks. In the first seven experiments the stretched and unstretched muscles were connected in series in one secondary circuit of an induction coil so that both muscles were stimulated simultaneously. The strength of the stimulus was that which called forth maximal response in the unstretched muscle. The stimuli were sent in at the rate of one per second. The direction of the current in the secondary circuit was varied so that in some experiments the stretched muscle and in others the unstretched muscle was first traversed by the current.

In the remainder of the experiments the two muscles were stimulated separately. It made no difference in the results whether the unstretched muscle was stimulated first or allowed to hang in the moist chamber until after the intervals of stretching and stimulation of the other muscle. For these experiments the strength of the stimulus was approximately the maximal break shock. Exact determination was not made to avoid delay, our judgment being based upon previous experience. The stimulus was of course the same for both muscles of a pair. The minimal stimulus for each muscle was determined in some of the experiments.

The stimulation of the stretched muscle was always carried out immediately upon the removal of the weight. The muscle was always left attached to the muscle lever and sometimes additional 20 or 30 grams were suspended from it. The unstretched muscle was connected to the muscle lever just prior to its stimulation and a load of 20 or 30 grams added if necessary to make the conditions uniform. Usually the writing point of the muscle lever was brought against the smoked

surface of the kymograph and the contractions recorded. The time required to effect complete exhaustion of each muscle was determined with a stop-watch. The results of these experiments are set forth in table 1.

TABLE 1

EXPERIMENT NUMBER	STRETCHING FORCE	DURATION OF STRETCHING	MINIMAL STIMULAS DISTANCE OF SECONDARY COIL FROM PRIMARY		LOAD	FATIGUE TIME	
			Stretched muscle	Unstretched muscle		Stretched muscle	Unstretched muscle
	grams	*minutes*	*cm.*	*cm.*	*grams*	*minutes*	*minutes*
1	100	30			20	17.76	20.15
2	100	30			20	14.08	19.50
3	100	30			30	4.86	6.06
4	100	30			30	3.25	5.41
5	100	30			None	10.40	10.40
6	100	30			None	16.86	17.25
7	100	30			None	14.96	15.75
8	100	30			20	18.50	20.50
9	100	30			30	15.36	15.36
10	100	30			20	11.51	16.70
11	100	60			20	12.63	17.36
12	100	60	21.6	15.7	30	11.76	10.20
13	100	90	18.5	22.8	None	27.08	38.83
14	100	45	20.5	19.5	None	38.75	46.88
15	100	45	21.9	22.4	20	22.75	25.05
16	100	120	25.1	26.3	20	13.40	10.55
17	100	120	18.5	21.7	30	13.30	11.75
18	100	120	19.3	21.2	None	38.66	46.16
19	100	165	23.6	19.6	None	25.15	19.50
20	100	195	26.1	27.1	20	10.08	13.66
21	100	60	23.3	19.8	None	10.08	10.76
22	100	180	19.5	17.3	20	16.41	17.93
23	100	150	21.1	24.5	None	16.08	19.11
24	100	120	21.4	19.7	None	20.16	37.08
25	100	140	28.0	20.1	20	9.63	9.35
Averages			22.0	21.2		16.53	19.25

We find that the average time for complete exhaustion of the muscle which had been extended by a weight of 100 grams for periods of 30 minutes to 3 hours was 16.53 minutes, and of the unstretched muscle which had been kept in moist air for similar periods of time was 19.25 minutes; that is, the former was completely fatigued in 14.02 per cent less time than the latter. We find, by comparison of the experiments

in which the interval of stretching was only 30 minutes with the others in which the extending force was allowed to act longer, that the effect was to a slight degree increased by the longer periods of stretching. In the former group the stretched muscle was completely fatigued in 13.46 per cent less time than the unstretched muscle and in the latter group in 14.41 per cent less time. When each muscle was required to lift a load of 20 or 30 grams at each contraction the difference in the fatigue time was 11.14 per cent; when the muscles were not loaded during the time of stimulation the stretched muscle was exhausted in 16.66 per cent less time than the other.

These results would seem to be sufficiently marked to justify the conclusion that a previous stretching hastens the onset of fatigue in a muscle. This is not due simply to an alteration in the irritability of the muscle since the average minimal stimulus is slightly weaker for the stretched than for the unstretched muscle. Obviously there is no opportunity for the isolated muscle to replenish its store of energy-yielding material and the using up of some of this by the muscle in combating the stretching force would hasten the development of fatigue since fatigue is not only a matter of accumulation of waste products but also an exhaustion of energy-yielding material.

CONCLUSIONS

1. The gastrocnemius muscle of the frog which has been stretched by a weight of 100 grams for periods of 30 minutes to 3 hours is completely exhausted by subsequent stimulation in less time than an unstretched muscle kept in moist air for similar periods of time.

2. This difference is increased slightly by increasing the duration of the stretching.

3. The stretching does not uniformly affect the irritability of the muscle.

4. It is suggested that the muscle uses up some of its available store of energy-yielding material in combating the stretching force and that the development of fatigue is hastened for this reason.

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EXTENSIBILITY OF MUSCLE: THE PRODUCTION OF CARBON DIOXIDE BY A MUSCLE WHEN IT IS MADE TO SUPPORT A WEIGHT

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Received for publication February 5, 1921

In a previous paper (1) we showed that the gastrocnemius of the frog, when stretched by a weight of 100 grams for ½ to 3 hours, can be completely fatigued in 85.98 per cent of the time required in the case of an unstretched muscle, when both are stimulated by the same strength induced current. We suggested that this effect of stretching might result because the muscle uses up some of its available reserve of energy-yielding material in combating the extending force, and that the muscle, to a certain extent at least, puts forth an active effort to support the weight. One would expect such an effort to involve the consumption of oxygen and production of carbon dioxide. The following experiment is indirect evidence of oxygen consumption under these circumstances.

We arranged two moist chambers with air-tight covers and provided circulation of oxygen through one and of nitrogen through the other. The flow of gases was regulated so as to be uniform in rate and pressure in the two chambers. Both gastrocnemii of a pithed frog were prepared and fastened in the femur clamps, one in the oxygen chamber, the other in the nitrogen chamber. After the flow of gases had been established and time allowed for air displacement in the apparatus, both muscles were loaded with equal weights and the amount of stretching of each in equal periods of time recorded. In six such experiments the actual lengthening of the muscle in oxygen averaged 4.05 mm. and of the muscle in nitrogen 5.08 mm.; the latter had stretched 25.43 per cent more than the former. Deprived of oxygen, therefore, the muscle is less resistant to the stretching force. Similarly Brodie (2) found that muscles in situ with intact nerve and blood supply were more resistant to stretching than isolated muscles. It is also true that in our experiments and in those of Brodie where the extension was greatest the recovery on removal of the weight was least perfect.

Gotschlich (3) in 1894 investigated the possible production of lactic acid by a muscle when it is stretched. He allowed weights of 100 to 1000 grams to extend one gastrocnemius of the frog (the other muscle was used as a control) for 1 hour. He then made muscle extracts in physiological saline solution and examined these extracts for lactic acid using the sodium salt of alizarin as indicator. He found uniformly no evidence of lactic acid in the unstretched muscle. He always found lactic acid in the muscle which had been stretched, the amount present being roughly proportional to the stretching force. It was also increased when the extension was effected at a higher temperature, 25°C. as compared with 11°C. When the load was 1000 grams, extending the muscle for 1 hour, he reports the detection of 1.69 mgm. of lactic acid per gram of muscle. Gotschlich says, "With maximal load and a temperature of 25°C. the quantity of acid was almost as great as that produced by a series of two hundred maximal contractions." He observed also that the acidity was still further increased by rhythmical loading and unloading over that resulting from constant stretching.

Garrey (4) notes that muscles which have been stretched increase in weight more rapidly than unstretched muscles when immersed in physiological saline solution. The difference is particularly marked when large weights are allowed to act for considerable periods of time. Fletcher (5) has shown that fatigued muscles, similarly immersed, increase in weight by the taking up of water more rapidly than resting muscles. He explains this as due to increased osmotic pressure within the muscle on account of the breaking up of substances during contraction. Accordingly Garrey interprets his findings as indicating an increased osmotic pressure in the stretched muscle from a like cause.

We have determined the amount of carbon dioxide produced in stretched and unstretched muscles by employing the principles of the apparatus devised by Tashiro (6), (7). The chief modification necessary for our purpose was an increase in the capacity of the apparatus. The services of a glass-blower were not available so that we have made use of such pieces of ready made glassware as were available in the laboratory. The set-up is shown in figure 1 and consisted in brief of the following parts:

A twelve liter bottle, *A*, containing 20 per cent solution of sodium hydroxide is connected through *1* with another twelve liter bottle, *B*, which holds at the beginning a small amount of 20 per cent sodium hydroxide solution. The air in *B* is vigorously shaken with the hydroxide solution, completely removing the carbon dioxide. By siphoning over more hydroxide solution from *A* this carbon

dioxide free air is driven out of *B* as required into the container, *D*, a 2,000 cc. flask. This flask is in siphon relation with the open wide-mouthed bottle, *C*, containing 5 per cent sodium hydroxide solution. Weak hydroxide solution is used so that the chamber will not be too dry and at the same time contamination of the carbon dioxide free air in *D* will be prevented. By means of *C* the air in *D* and in fact throughout the remainder of the apparatus is kept at atmospheric pressure. The chambers, *G* and *G'*, cylindrical separatory funnels, were provided with carefully fitted stoppers from the under surface of which the muscles were suspended. These stoppers were so arranged that they might be mercury sealed. Into each chamber was run a tube with upturned end, *X* and *X'*, to form a cup for the drop of barium hydrate solution. Connected with *G* and *G'* was a mercury reservoir, *H*, for regulating their capacity. Also connected with *G* and *G'* respectively were graduated burettes, *I* and *I'*, by which measured small quantities of

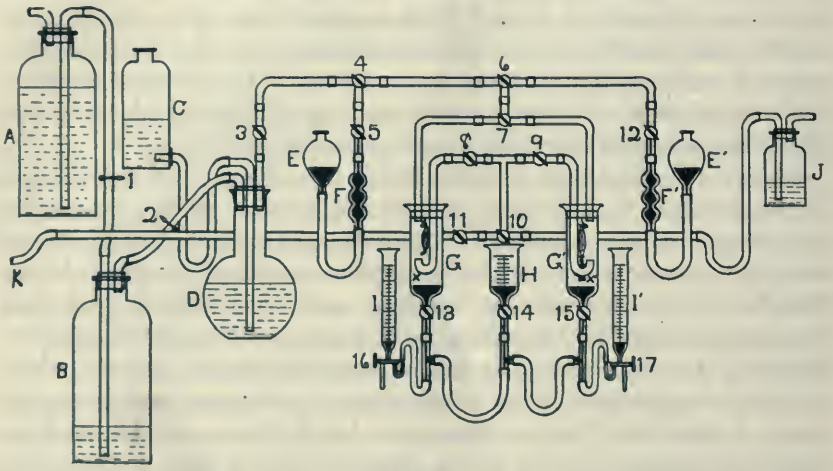

Fig. 1

mercury could be withdrawn as required in the quantitative determination of the carbon dioxide produced. The blood gas bulbs, *F* and *F'*, with the mercury pressure funnels, *E* and *E'*, were used for the withdrawal of air from *G* and *G'* and its subsequent replacement in the quantitative analysis. A bottle, *J*, containing saturated solution of barium hydrate was connected with the tubing terminating at *X* and *X'*, and an efficient electric vacuum pump was connected on at *K*. Heavy walled capillary glass tubing and stopcocks were used throughout, except the terminal part of the barium hydrate tubes, *X* and *X'*, where 3 mm. bore tubing was used. All rubber connections were made as short as possible and all joints were rendered air-tight by repeated coating with shellac.

The first step in the experimental procedure consisted in the testing of the apparatus for its air-tightness and the absolute freedom from carbon dioxide of the air supplied from *B*.

With the apparatus clean and dry stopcocks *4*, *6* and *7* were turned so as to make connection between *D* and *G*, *G'* only. Stopcock *3* was closed, stopcocks *8*, *9* and *11* were opened and stopcock *10* was turned to connect the upright tube with *11*, excluding the tube from *J*. The air was now exhausted from the chambers *G* and *G'* by the pump at *K*. Stopcock *11* was closed and stopcock *3* opened permitting carbon dioxide free air to enter from *D* to fill the vacuum created in *G* and *G'*. The pressure was made equal to atmospheric pressure by adjusting the level of *C*. This process of evacuating *G* and *G'* and refilling with CO_2-free air was always repeated at least four times. The efficiency of the apparatus depends very largely upon the thoroughness of this washing-out process. When *G* and *G'* finally contained pure CO_2-free air barium hydrate solution was let in from *J* by turning stopcock *10* in the proper direction (*11* was closed) and very gradually opening stopcocks *8* and *9* in turn until a hemispherical drop of clear barium hydrate solution stood on the upturned tubes at *X* and *X'*. Stopcocks *8* and *9* were closed, the pressure quickly adjusted to atmospheric pressure at *C* and stopcock *3* was closed. If the air is absolutely free of carbon dioxide the drops of barium hydroxide solution remain clear indefinitely. On the other hand, if carbon dioxide is present, even as little as 1×10^{-7} grams will cause precipitation within 10 minutes of particles of barium carbonate in the drop of barium hydrate and larger amounts will cause increase in the size and number of the particles (7). The precipitate is detected by observation of the drop through a magnifying lens.

In our apparatus the drop of barium hydrate obtained in the manner described remained clear over night in repeated tests, proving beyond question that the air in *B* was absolutely carbon dioxide free, that the washing out process was thorough and complete, and that there were no leaks.

Oxidation processes which would lead to the production of carbon dioxide have been shown by various investigators,—Herman (8), Hill (9), Gyllenswärd (10)—to occur in the surviving isolated muscles of the frog. To prove liberation of carbon dioxide in a muscle under the influence of a stretching force it is necessary to demonstrate, therefore, that such a muscle produces carbon dioxide more rapidly or in greater amount than a muscle of the same size under the same conditions of temperature, atmospheric pressure and surrounding medium. It was for a comparison of this sort that the two chambers, *G* and *G'*, were provided. It was further necessary for an exact comparison to have the capacity of the two chambers identical. For this purpose both chambers were stoppered in the usual manner after placing in one the weight to be subsequently used to stretch the muscle (an ordinary 50-gram weight with a wire ring attached). Then the chambers were completely filled with mercury from *H*, exactly 150 cc. of mercury were withdrawn from each and the stopcocks *13*, *14* and *15* closed, leaving a safety

layer of mercury in the bottom of each chamber. The gas capacity of G was, therefore, exactly equal to the gas capacity of G' before the introduction of the muscles.

Both gastrocnemii of a pithed frog were prepared in the usual manner, care being exercised to cut the tendon, the tibia and the femur at the same points in both preparations. Except in a very few of the early experiments all the muscular tissue about the knee joint except the gastrocnemius was carefully dissected away. An S-shaped pin-hook was passed through the knee joint and another through the tendon of each preparation. Each muscle was hung from a hook on the under surface of the stopper of its chamber; the 50-gram weight was hung from one of the muscles; the stoppers were put into position and sealed. The air was exhausted and G and G' refilled with CO_2-free air by the washing out process described in connection with the testing of the apparatus. The drops of barium hydrate were got into position; pressure in various parts of the apparatus was equalized by adjustment at C; and very careful watch was kept for the first appearance of precipitate in the barium hydrate. Twenty-one such observations were made.

Averaging the results of these experiments we find that 4.07 minutes elapsed between the placing of the drop of barium hydrate in position in the chamber containing the stretched muscle and the first appearance of precipitate, whereas the interval was 17.47 minutes in the case of the unstretched muscle. In the individual experiments the results varied between 2 and 10 minutes for the stretched muscle and between 10 and 30 minutes for the unstretched muscle. In every instance the drop of barium hydrate in relation with the stretched muscle showed precipitate much sooner than that in relation with the unstretched muscle.

As a further check the use of the chambers was alternated from time to time with proper volume correction. Since we used companion muscles from the same frog it seems justifiable to disregard any slight differences there might have been in the two muscles under identical tension conditions. In nearly all cases, as pointed out, all muscular tissue other than the gastrocnemius was dissected away avoiding any variation from this source. Theoretically the surface area of the muscle might be increased by stretching and with increased surface area there might be greater diffusion outward of carbon dioxide without necessarily increased production. But actual measurement showed that there was sometimes no change at all and at other times a slight, usually insignificant, increase in surface area.

We have made but one quantitative determination of the carbon dioxide produced by the stretched and unstretched muscles, again following the method described by Tashiro (7).

With stopcock *3* closed and all the other stopcocks turned to make connection between *F*, *F'* and the tube to the pump, *E* and *E'* were raised until a bit of mercury was forced from each direction into the capillary tube between stopcocks *4* and *6*. Stopcocks *5* and *12* were now closed and stopcocks *4* and *6* turned cutting off the connection to *F* and *F'* respectively. *E* and *E'* were kept elevated so that the mercury in them was at least up to the level of the upper horizontal tubing. The mercury in the tubing between stopcocks *4* and *6* was displaced into *G*, *G'* by greatly elevating *C* and was withdrawn into *I*, *I'* so as to restore the correct volume in the muscle chambers.

The two gastrocnemius muscles were prepared as in the other experiments, placed in position in the chambers *G* and *G'* with the 50-gram weight suspended from the muscle in *G'* and the washing out process gone through with in the way already described. At the expiration of 60 minutes stopcocks *4*, *5*, *6* and *7* were turned to make connection between *G* and *F* only. Then, stopcocks *13* and *14* having been opened, by raising *H* mercury was run into *G* and the air displaced into *F*. It was of course necessary to lower *E* at the same time sufficiently to maintain atmospheric pressure. After the expulsion of all of the air in *G* stopcock *5* was closed, mercury was again withdrawn from *G* to restore its volume to 150 cc. and stopcock *13* was closed. In like manner the air in *G'* was transferred into *F'*. The muscles were removed, the stoppers replaced and sealed, the washing out process repeated and drops of barium hydrate solution got into position at *X* and *X'* when the chambers finally contained CO_2-free air. The pressure was made equal to atmospheric pressure by adjustment at *C*; stopcocks *3*, *8* and *9* were closed. Stopcocks *4*, *5*, *6* and *7* were turned to make connection between *F* and *G* (the chamber which had contained the unstretched muscle). By opening stopcock *13* and turning stopcock *16* at the bottom of the graduated cylinder *I*, measured volumes of mercury were withdrawn from *G* permitting air to enter from *F* with readjustment of pressure by raising *E* as necessary. In this manner 5 cc. of air were transferred from *F* into *G*. Ten minutes later, the drop of barium hydrate being still clear, another 5 cc. of air were transferred into *G*. We continued to transfer 5 cc. of air at a time at 10-minute intervals until the thirteenth instalment (a total of 65 cc.) was introduced. Three minutes later precipitate began to form at *X*. By the same method air was transferred from *F'* to *G'* (the chamber which had contained the stretched muscle) in 5 cc. increments at 10-minute intervals. Three and a half minutes after the introduction of the third 5 cc. increment, precipitate first appeared at *X'*. The quantity of air containing the minimum amount of carbon dioxide which will precipitate barium carbonate when diluted by 150 cc. of CO_2-free air was, therefore, approximately 65 cc. when using air in which the unstretched muscle had been suspended and 15 cc. when using air in which the stretched muscle had been suspended. To arrive more closely at the minimum quantity of air required to produce a precipitate in the two cases the capacity of each of the chambers was restored to 150 cc. by introduction of mercury from *H*, the washing-out process

was repeated and drops of barium hydrate again got into position at X and X'. Sixty cubic centimeters of air were run in at once from F to G by removing mercury into I. Ten minutes later the drop was still clear. Then 1 cc. of air at a time was introduced at ten minute intervals. Six minutes after the introduction of the second 1 cc. increment precipitate began to form at X. Ten cubic centimeters of air were transferred from F' to G' and 1 cc. at a time at 10-minute intervals until precipitate began to form at X', 2 minutes after the introduction of the fourth cubic centimeter. Therefore, 62 cc. of air from the chamber which had held the unstretched muscle and 14 cc. of air from the chamber which had held the stretched muscle each contained the minimum quantity of carbon dioxide necessary to cause a precipitate in the drop of barium hydrate, when added to 150 cc. of CO_2-free air.

According to Tashiro (6) the minimum quantity of carbon dioxide which will cause a precipitate to form within 10 minutes in the drop of barium hydrate is 1×10^{-7} grams when the total volume of the chamber in which the analysis is made is 15 to 25 cc. In our experiments, where the total volume of the analysis chamber was 150 cc., the minimum carbon dioxide value will be at least as great, possibly greater. Using Tashiro's figures, however, as equivalent to the 62 cc. in the one case and 14 cc. in the other, we calculate the total carbon dioxide produced by the unstretched muscle in 60 minutes as $\frac{150}{62} \times 10^{-7}$ or 2.4×10^{-7} grams, and by the stretched muscle in the same period as $\frac{150}{14} \times 10^{-7}$ or 10.7×10^{-7} grams; the latter had produced 4.45 times as much as the former.

CONCLUSIONS

1. The isolated gastrocnemius muscle of the frog when extended by a weight of 50 grams produces carbon dioxide at a faster rate than an unstretched muscle otherwise under the same conditions.

2. In a 60-minute period the stretched muscle produces about four and a half times as much carbon dioxide as the unstretched muscle.

3. The increased carbon dioxide production is direct evidence that the stretched muscle puts forth an active effort to support the weight.

The experiments herein described were carried out chiefly in the Department of Physiology of McGill University and our thanks are due Prof. John Tait for the courtesies extended.

We wish to acknowledge our indebtedness to Mr. Charles A. Eddy for making the diagram which accompanies this report.

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STUDIES OF THE THYROID APPARATUS

I. The Stability of the Nervous System as a Factor in the Resistance of the Albino Rat to the Loss of the Parathyroid Secretion

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Received for publication February 10, 1921

This paper is a report and an interpretation of the differences of resistance of two groups of albino rats to the loss, by operation, of the parathyroid secretion. No general review of previous studies of the parathyroids is given and reference is made only to the landmarks and investigations pertinent to the present subject, since that mass of material has been thoroughly covered by other workers (1).

Gruber (2), in 1845, was probably the first to call attention in the literature to those little groups of cells anatomically associated with the thyroid gland which we now know as the parathyroids from the name given to them by Sandström (3) in 1880, and around the function of which in connection with thyroid function there raged that vivid controversy between Gley (4) and Moussu (5). Later work has established definitely that the parathyroids do possess a distinct function independent of that of the thyroid. Whether or not the parathyroids possess functions that are complementary, supplementary or antagonistic to any thyroid functions is not germane to the present discussion.

The discovery of the parathyroid glands in the albino rat can be credited to Cristiani (6), who reported that this animal possessed two of these glands, one associated with each of the two lobes of the thyroid; thus distinguishing it from many other mammalia, most of which have four. Although Erdheim (7) claimed to have found cellular groups, which in his opinion are to be considered as accessory parathyroids, in various places in the neck and in the upper pole of the thymus, yet he hedges by stating that these so-called accessory glands are always very small and only a minute part of the total parathyroid tissue and do

not seem to take part in any function of the gland. Moreover, he fails to present any plates showing the histological structure of these cell groups as supporting his contention that they are parathyroid tissue. It has been suggested that these small groups of cells could as well be thymic residues as accessory parathyroids. Later studies by Vincent and Jolly (8) and Thompson (9) sustain the earlier findings that the rat has but two parathyroid glands. I have carefully examined with a hand lens the neck and thymus of my operated animals and have yet to find any tissue that could be designated as parathyroid tissue outside of that found in close association with the thyroid.

In 1884 Schiff (10) described in detail the symptoms of tetany and disturbances of the nervous system following the removal of the thyroid gland from dogs, thus correlating them with similar occurrences observed after strumectomy in man previously reported from the clinical side by Weiss (11) and Reverdin (12). Since that time there have been many other descriptions of these phemonena, all phases of which I have seen in my operated rats, and which are now known to be due to the loss of the parathyroid secretion and not to a thyroid disturbance or the mechanical effects of the operation.

Inasmuch as the symptoms occurring after the removal of the parathyroids point unmistakably to an involvement of the nervous system in the final result, various workers have attempted to find histological evidences of alterations in the different parts of the nervous system of animals dying in parathyroid tetany. Much contradictory evidence is given. Awtokratow (13), Sanquirico and Canalis (14), de Quervain (15) and others could find no constant changes in the nerve elements sufficient to justify the opinion that anatomical alterations of cells or fibers are factors in the expression of parathyroid tetany. On the other hand Capobianco (16), Vassale and Donaggio (17), Maas (18), Lupó (19) and others report the presence of intensive degenerative changes in various parts of the nervous system. Unfortunately, however, but little agreement is found among the investigators as to the exact part of the nervous system attacked; though all agree that the tetany observed is due to an intoxication of the nervous system. These discrepancies are explicable from the fact that the specimens examined varied in the length of time they had been in the fixing fluid from a few days to a year or more; from the fact that the different workers failed to use the same method of fixation and staining; and from the fact that their specimens were taken from animals dying after thyreo-parathyroidectomy or parathyroidectomy at intervals from a few days to several weeks.

In 1912 Möllgaard (20), appreciating that a probable cause of these differences lay in the technique of fixation, made a histological comparison of the motor cells of the cord, the cells of the spinal ganglia, and the motor cortex of normal dogs and of dogs dying in parathyroid tetany. He used for his study sections cut from fresh frozen specimens. He found no changes in the motor cortex and no degenerative changes anywhere. He did find, however, that there were cells in the cord and spinal ganglia of the parathyroidectomised animals that differed from the normal in that no network could be brought out on staining. He therefore concluded that the cause of the parathyroid tetany and convulsions is an alteration of the physical condition of the nerve cells due to some toxic substance. This is supported from the physiological side by the experiments of Paton, Findlay and Watson (21) which they interpret as showing that the nervous symptoms are due to the condition of the central nervous system; that the cerebral arc is not directly involved; and that the electrical excitability of the peripheral nerves is increased when any marked decrease in parathyroid tissue has been produced.

Hence the evidence that the death of the parathyroidectomised animals is due to an immediate degenerative effect on the nerve elements of a toxic substance, either produced as a result of a perverted metabolism following the lack of the parathyroid secretion, or which is a normal product of neuro-muscular metabolism and is now uncompensated for by destruction or neutralization through the mediation of the parathyroid secretion, is quite generally negatived. This is particularly true from the fact that many of my parathyroidectomised rats exhibited tetany paralysis and convulsions within an hour and a half, and died in convulsions within three or four hours after the operation. The possibility of marked degenerative changes having occurred in the nervous system in vivo in this short period is rather dubious. This, however, does not exclude the possibility that through a long-continued action of the parathyroid deficiency there may be produced degenerative changes in the nerve elements by virtue of the repeated action of small amounts of the unknown constituent which in larger amounts produces acute tetany and death. In connection with the idea that these nerve disturbances are due to some toxic substance, attention should be called to the fact that although Paton and Findlay (22) consider that the idea of the parathyroid acting as "detoxicating some normal product of metabolism as hardly worthy of consideration" yet they base their theory of idiopathic and parathyroid tetany on the

development of some "guanidine-like body" in these conditions and state that "the parathyroids thus control the metabolism of guanidine in the body by preventing its development in undue amounts." This to my mind is a distinction without a difference.

From the reports of Cramer (23) and his collaborators, Gies (24) and his collaborators, Kojima (25) and Larson (27), it is evident that the rat can survive thyreo-parathyroidectomy or parathyroidectomy for some time without indications of an acute involvement of the nervous system, thus disproving Cristiani's (27) statement that complete thyroidectomy is always fatal. These observations lead one to suspect that either the nervous system of the survivors is different in its powers of resistance to the toxemia from that of those which die, or that the cause of the disturbances is not present in sufficient concentrations at any one time to produce acute symptoms.

My interest was attracted to this particular phase of the subject by the fact that although frequent deaths occurred from parathyroid tetany in my thyreo-parathyroidectomised rats, yet some of the operated animals did not die.

The general technique of removal of the thyroid apparatus was no different from that described by Cristiani (6) and others; but when the parathyroids were alone removed they were cut out of the thyroid with sharp, fine-pointed scissors. Hemorrhage was prevented by stopping off the superior thyroid arteries with small clamps made from ordinary paper-clips bent into appropriate shape and having the opposed ends flattened.

On examination it was found that the operated animals had come from two separate groups of rats, and that the greater percentage of survivals had occurred in the group that came from the so-called "Experimental Colony" of the Wistar Institute. We have here at the Wistar Institute two colonies of albino rats, among others, both of which are the descendants of the same original pair. One of these colonies, that which is called the Experimental colony, has been petted and gentled, while the other, the Standard stock, has had only that human contact incident to routine feeding and cage-cleaning. The behavior of the rats in this latter group is that of the ordinary laboratory animal. They are timid, apprehensive and high-strung. When picked up they are tense and resistant, frequently exhibiting their natural defensive instincts of fear and rage by biting. *The picture as a whole is one of constant high irritability and neuro-muscular tension.* It is of course not impossible that the high grade of emotional tension ever

present in the rats that have not been gentled contributes to the general picture of high tension through mediation of the adrenals.

The behavior of the gentled group is in marked contrast to that of the others. In these animals the defensive instincts have been repressed by the constant handling and petting. This gentling has been carried on for the last five generations. When the animals are picked up they are relaxed and yielding. They are not easily frightened. *They give a uniform picture of placidity. The threshold of the neuromuscular reactions to potentially disturbing stimuli is almost prohibitively high.*

We now turn to the actual experimentation carried out in order to determine whether or not there was a difference in the susceptibility of the animals from the two groups to the loss of the parathyroid secretion.

A total of 304 rats was operated. Both sexes were used in this study and the ages varied from 30 to 100 days. The majority of operations, however, were done on rats around 55 days old.

It has been noticed in the preliminary work that if the rats did not develop tetany or convulsions and die within 48 hours then they probably would continue to live for a considerable period thereafter. In fact the majority of survivals of the 48-hour period lived until they were 150 days old when they were killed for examination. Consequently it was evident that the fatalities from acute parathyroid tetany would usually occur in less than 48 hours. This period then was taken as the standard of probable survival.

Complete thyroidectomy, which included the removal of the parathyroids, was done on 90 rats from the Standard stock consisting of rats that had not been gentled. Out of these 79 per cent died in parathyroid tetany within the 48-hour period. During the same period 96 rats from the Experimental colony, the gentled stock, were similarly operated. The survivals from this series were many more. Eighty-seven per cent lived and but 13 per cent died. Among a series of rats from the Standard stock 28 were thyreo-parathyroidectomised in two stages. One-half of the thyroid apparatus was removed at the first operation and the second half was removed two weeks later. The mortality in this group was 68 per cent, or slightly lower than that of the first lot recorded. The lower mortality is explicable by the fact that during the interval some of the rats had become slightly less tense, though no special attempts had been made to gentle them.

In order to demonstrate that the results were not due to the loss of the thyroid but were due solely to the removal of the parathyroids, 51 rats from the Standard stock were parathyroidectomised. Within less than 48 hours 76 per cent of them had died. When 31 rats from the gentled stock were parathyroidectomised but 13 per cent of them died. The almost exact coincidence of these ratios with those of the thyreo-parathyroidectomised groups is conclusive evidence that we are here dealing with a parathyroid loss alone as the causative factor in the fatalities.

I next removed 28 animals born of the gentled stock from their mothers at weaning and put them, until they were 55 days old, under the same conditions of environment as the rats in the Standard stock. They were not gentled. At the same time a like number of rats from the Standard stock were removed from their mothers at weaning and set aside under their usual environmental conditions for thyreo-parathyroidectomy at 55 days of age as controls. The usual 78 per cent of fatalities occurred in the Standard rats. However, much to my surprise, when the progeny of the gentled rats were brought to me they were almost as tame as their parents and when completely thyroidectomised but 14 per cent of them died. This rather indicates that the gentling process for three generations had resulted in the elimination of the defensive reactions of the young with a consequent stabilization of the nervous system and lowered tension which had been passed along.

A final study was then made in which one-half of each of several litters of rats from the Standard stock were placed in the Experimental colony at weaning and gentled, while the other half was to have been kept under the usual conditions of Standard room care and not gentled. As controls the same number of animals from several litters of the gentled stock were similarly split, one-half of which was put under the Standard stock conditions and the other half kept and gentled as usual. When this series was brought to the laboratory for operation at the age of 55 days they all, those from the Standard stock as well as those from the gentled stock, appeared tame, non-resistant and relaxed. Acute tetany did not appear within the 48-hour period in any of these rats after the loss of the parathyroid secretion by operation. This definitely showed that the stability of the nervous system is a strong factor in the resistance of albino rats to the loss of the parathyroid secretion. A study of the records of the sex, body weight and body length of all the lots operated showed that these factors could not be considered as playing any significant rôle in the final results. Nor could any dietary

differences of either qualitative or quantitative nature be invoked as contributing causes. The only observable differences in the two groups of operated animals are their nervous reactions to the environment as already described. That such differences have been shown to exert an influence on metabolic stability has already been indicated (28). That they should result in such marked differences in dependency on the parathyroid secretion is remarkable and affords conclusive evidence that a condition of nervous stability is conducive to a greater resistance to the loss of the parathyroid secretion than is a condition of high-strung irritability.

In view of the striking differences obtained a brief attempt at interpretation seems justified, based on related data from other sources. It would be a gratuitous assumption to consider that the differences in mortality in the two groups were due to differences in grade of parathyroid secretion, since we have no direct indication of this latter possibility. Yet if the condition of hypertension in the rats that had not been gentled is to be considered as a result of a diminished parathyroid secretion, the condition of stability shown in the gentled group should *pari passu* be considered as due to a relatively greater parathyroid secretion. If such is the case this would imply a natural or acquired greater dependency on such secretion and hence more serious defection on its removal. This we have seen is not the case.

Now it is a well-known fact that excitability tends to the production of a condition of heightened muscular tone, while calmness or lack of emotional strain results in a lower tension. This simply means that the metabolic changes taking place incident to the maintenance of muscle tone are of a lower order of magnitude in gentled animals than they are in rats that have not been gentled. Such being the case, let us assume as a working basis that the normal function of the parathyroid is to prevent the accumulation of or to cause the catalytic destruction of any tetany-producing substance such as ammonia, which MacCallum and Voegtlin (29) have shown to be excreted in more than normal amounts in parathyroid tetany; which Carlson and Jacobson (30) showed to cause tetany; and which Tashiro (31) has demonstrated to be a product of the tetanized nerve-muscle preparation; or of guanidine, which Burns and Sharpe (32) have found to be increased in the blood and urine of parathyroidectomised dogs, and which Paton and Findlay (22) have shown to produce symptoms identical with those produced by parathyroid tetany. Then in either case, whether these substances are abnormal by-products from the metabolism of muscle tone due to the lack of the regulating effect of the parathyroid secretion

on that phase of muscle metabolism, or whether they are normal products of this reaction and are not neutralized or destroyed because of the lack of the catalyzing agent produced by the parathyroids, the explanation of the high mortality in the group of rats of high nervous tension can be based on the idea of an inherently greater tendency to the formation of the toxic compounds than is found in those rats of low muscle tone. When the parathyroids are removed from the latter type of animal the decrease in the catalytic effect is less destructive because less of the toxic compounds are normally produced and the animal continues to live because the body as a whole is able in some way to dispose of this smaller amount on the basis of the same general principle Kendall (33) has evolved for the vitality expressed in athyroid conditions.

SUMMARY AND CONCLUSIONS

A study is reported of the relative mortality after thyreo-parathyroidectomy and parathyroidectomy of two groups of albino rats, differing only in the stability of their nervous systems as evidenced by excitability and a high-tension reaction to the environment in the one group, designated as Standard stock, or rats that had not been gentled; and by a condition of relaxation and high threshold toward potentially disturbing stimuli in the other or gentled group. In all, 304 rats were operated. Complete thyroidectomy resulted in a mortality by acute parathyroid tetany of 79 per cent of the Standard stock rats. The same operation in the gentled rats gave but a 13 per cent mortality. The same ratios were found to hold when parathyroidectomy alone was done. Gentled rats of the third generation when put from weaning under conditions of identical environment as those obtaining for the Standard group still showed but a 14 per cent mortality. Standard stock rats when gentled had their mortality rate reduced to zero in the relatively small series studied. No sex, size, weight or dietary variations can be used as explaining the differences in mortality of the two groups. For reasons explained in the text, 48 hours was taken as the standard period of survival in the comparisons.

It is therefore concluded that the condition of stability of the nervous system induced in albino rats by gentling and petting produces in them a marked resistance to the loss of the parathyroid secretion, which in excitable rats normally results in death from acute parathyroid tetany in less than 48 hours.

This is interpreted on the basis of our present knowledge to be possibly due to an inherently lesser production in the gentled rats of

certain known tetany-producing substances such as ammonia or guanidine as by-products of that phase of muscle metabolism concerned with muscle tone. Whether or not these toxic substances accumulate in the operated rats that have not been gentled because of an abnormal metabolism due to the loss of a regulating influence exerted by the parathyroids, or whether they are normal products of muscle catabolism and accumulate because they are not neutralized or destroyed by the catalytic action of the parathyroid secretion is a question calling for further study.

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STUDIES IN NUTRITION

VI. The Nutritive Value of the Proteins of the Lima Bean, Phaseolus Lunatus

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Received for publication February 11, 1921

The fact that the proteins of the navy bean, *Phaseolus vulgaris*, not only are deficient in cystine but also require cooking for a short time before they become biologically available has been shown in a recent publication from this laboratory (1). This suggested a line of attack upon the problem of the nutritive value of the closely related lima bean, *Phaseolus lunatus*. At the time that the paper on the navy bean was published the question as to what effect cooking had upon the proteins was left in abeyance. It has since been shown (2) that the difference due to cooking is to be explained, at least in part, to an increase in digestibility.

In the present paper it is shown that the lima bean when cooked, dried, supplemented with cystine, and fed to albino rats as the sole source of protein in an otherwise complete diet produced normal growth. The animals fed a similar diet without the addition of cystine merely maintained their weight. Those receiving a diet of raw lima bean meal, however, made no growth, even if cystine was added to complete the diet.

Experiments with raw and cooked lima bean meal. A diet was prepared which contained 75 parts of raw lima bean meal, 11 parts of lard, 10 parts of butter fat and 4 parts of an inorganic salt mixture.[1] A similar diet was made with lima bean meal which had been cooked and dried. Both of these diets contained 15 per cent of protein. The rats fed either of the diets made no growth, but were able to maintain their weight. The growth curves are shown on chart 1.

[1] The composition of the salt mixture used in the diets described in this paper is the same as that used by Osborne and Mendel (3) in their experimental diets.

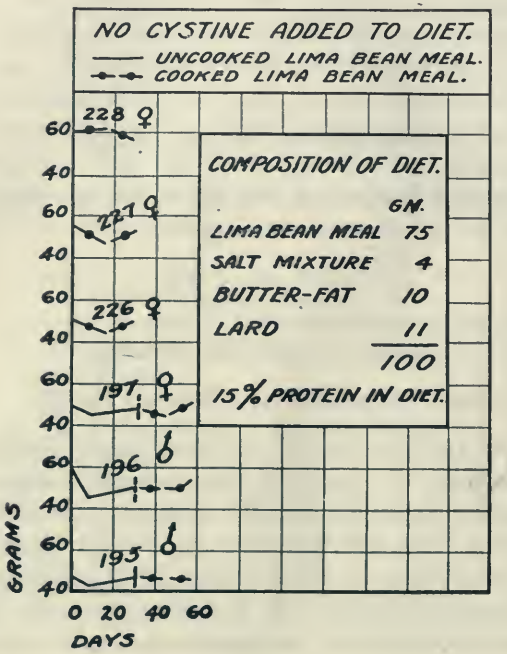

Chart 1

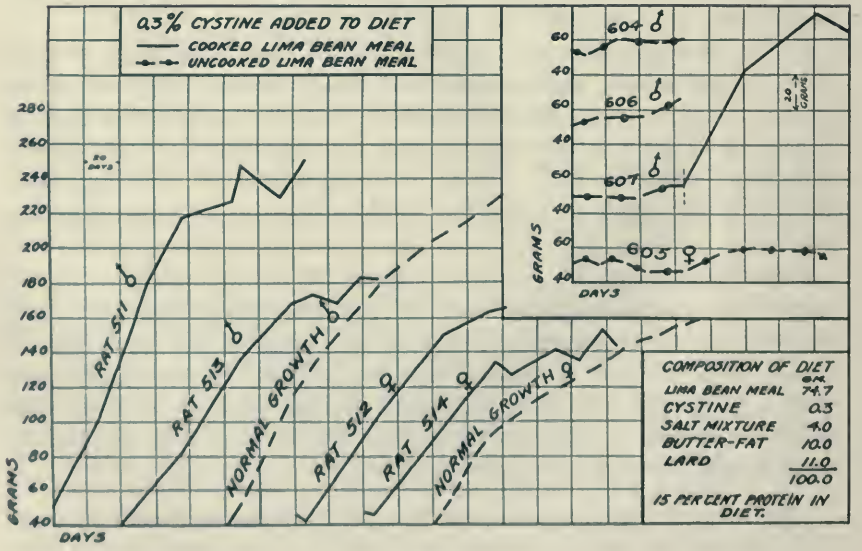

Chart 2

Experiments with raw and cooked lima bean meal supplemented with cystine. Two diets were prepared, one from cooked and the other from uncooked lima bean meal, to both of which 0.3 per cent of cystine was added. To complete these diets, each of which contained 15 per cent of protein, butter fat, lard and an inorganic salt mixture were added. The animals receiving the cooked lima bean meal supplemented with cystine grew normally, while those fed the raw lima bean meal and cystine diet merely maintained their weight. These experiments indicate that the lima bean meal, after it had been cooked and dried, contained sufficient water-soluble vitamine. Rat 607♂ gained very little in weight the first 65 days, during which it received the raw lima bean diet supplemented with 0.3 per cent cystine. At the end of this period it was given a similar diet with the exception that the lima bean meal had been cooked. The animal then at once resumed the normal rate of growth. The composition of the diets and the growth curves are recorded on chart 2.

SUMMARY

A diet of cooked lima bean meal supplemented with 0.3 per cent of cystine, together with the other necessary non-protein dietary ingredients, furnished adequate protein for the normal growth of albino rats. A similar diet, to which no cystine was added, merely maintained the weight of the experimental animals. Growth did not occur if the diet consisted of either raw or cooked lima bean meal which was not supplemented with cystine although the other non-protein dietary factors were added.

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STUDIES IN NUTRITION

VII. The Nutritive Value of the Proteins of the Adsuki Bean, Phaseolus Angularis

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Received for publication February 11, 1921

The adsuki bean, *Phaseolus angularis*, has long been used as food by the people of the Orient. Its varieties are very numerous and are distinguished principally by their period of maturity, together with the color of seeds and pods. The seeds are subcylindric with ends a little flattened and slightly longer than broad and the embryo in all cases nearly white and brittle in consistency.[1]

Nutritive studies with the navy and lima beans, which belong to the same botanical genus, have already been published from this laboratory (1), (2). The proteins of these two seeds lacked two factors before they became available for the normal nutrition of albino rats. First, a deficiency of cystine, which was corrected by the addition of 0.3 per cent of this amino acid to a diet adequate in other respects. Second, cooking, without which growth could not be obtained. In the case of the adsuki bean, however, while it was found that its proteins were deficient in cystine, cooking was not required.

Experiments were made with the isolated adsuki globulin as well as with raw and cooked adsuki bean meal. When the isolated protein,[2] together with the other non-protein dietary essentials, was fed to albino rats they grew at about two-thirds of the normal rate. If, however,

[1] The beans used in the experiments described in this paper were furnished by the Bureau of Plant Industry, United States Department of Agriculture, and were the maroon variety. A complete description of the adsuki bean is given by C. V. Piper and W. J. Morse in U. S. Dept. Agric. Bulletin 119, 1914.

[2] The adsuki protein was prepared by precipitating a clarified 1 per cent sodium chloride extract of adsuki bean meal in 5 volumes of distilled water. The protein thus obtained was washed until free from chlorides and the water removed with absolute alcohol and absolute ether. Unpublished experiments by Chas. E. F. Gersdorff of this laboratory indicate that the adsuki protein so prepared is a mixture containing two globulins differing in their sulfur content.

the protein was supplemented with cystine, the rate of growth was markedly increased.

A diet of either raw or cooked adsuki bean meal and the other necessary dietary ingredients enabled albino rats to grow at only about one-third to two-thirds of the normal rate. If, however, 0.34 per cent of cystine was first added to either the raw or cooked bean meal, the rats grew at the normal rate. The growth obtained with the raw adsuki bean meal supplemented with cystine was surprising in view of the fact that neither the navy nor the lima bean produced growth, even when this factor was added.

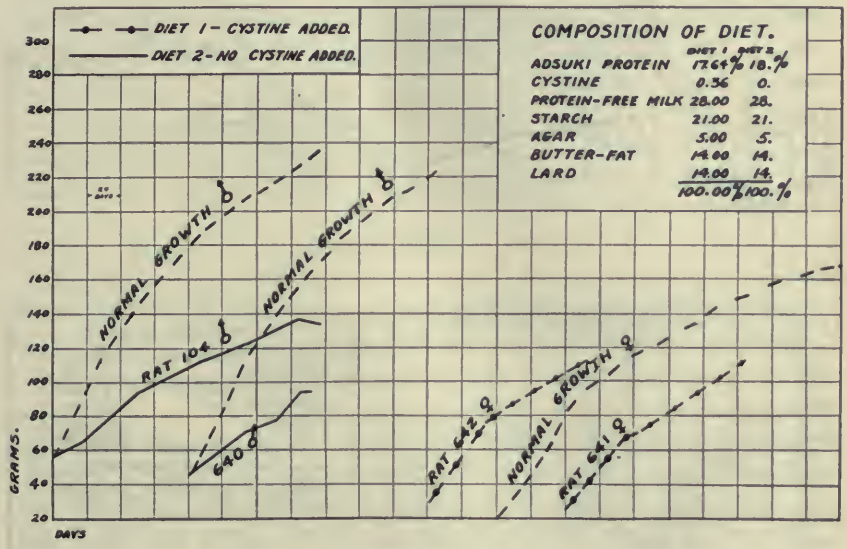

Chart 1

Experiments with the isolated adsuki protein. Two diets were prepared from the adsuki protein, in one of which the protein was supplemented with 2 per cent of cystine. The water-soluble vitamine and inorganic salts were furnished by protein-free milk, while butter fat gave the necessary fat-soluble vitamines, and the diet was made adequate in other respects. Rats 641 ♀ and 642 ♀ were placed on diet 1, which contained cystine. These animals grew at almost the normal rate. Diet 2, which had not been supplemented with cystine, was fed to rats 104 ♂ and 640 ♂ and the rate of growth was from one-third to one-half of the normal. The composition of the diets and the growth curves are recorded on chart 1.

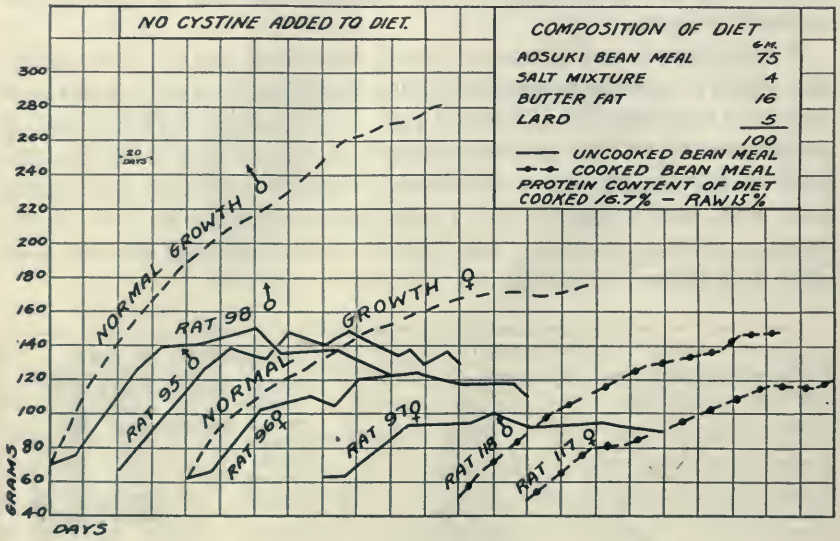

Chart 2

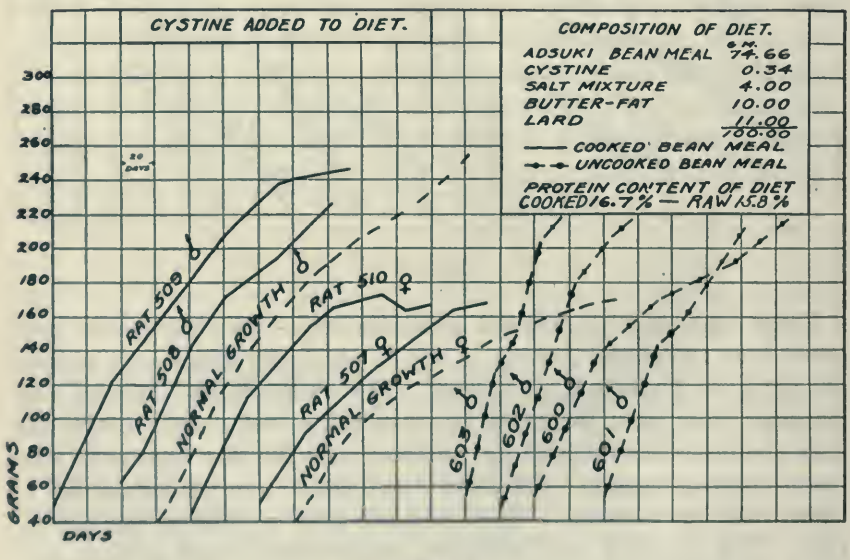

Chart 3

Growths experiments with raw and cooked adsuki bean meal. Diets were prepared from both the raw and cooked bean meal, to which was added an adequate inorganic salt mixture,[3] butter fat and lard. These diets enabled the rats to grow at only from one-third to two-thirds of the normal rate. The composition of the diet and the growth curves are shown on chart 2.

Growth experiments with raw and cooked adsuki bean meal, supplemented with 0.34 per cent of cystine. Rats grew at the normal rate when fed a diet similar to that described above, except that 0.34 per cent of cystine replaced an equivalent quantity of the bean meal. It is interesting to note that the raw bean meal, when supplemented with cystine, was as efficient in producing normal growth as the cooked meal. These results are shown graphically on chart 3.

TABLE 1

*Average gain of body weight per gram of ingested protein. 4-week period**

DIET	COOKED ADSUKI BEAN	COOKED LIMA BEAN	COOKED NAVY BEAN	COOKED PHASEOLIN†	RAW ADSUKI BEAN	RAW LIMA BEAN
Gain per gram of protein ingested (*grams*)	1.5	1.2	1.5	1.5	1.7	0.16
Protein in diet (*per cent*)	16.7	15	18	20	15.8	15

* Cystine was added to all diets.

† Isolated navy bean protein.

Relative efficiency of the proteins of the genus Phaseolus. The weight of food ingested was determined each week and compared with the rate of growth. The data given in the following table show that there was very little difference in the gain in weight made by the animals for each gram of protein eaten when the beans were cooked and properly supplemented with cystine. The raw proteins of the adsuki bean were as efficient as the cooked for normal growth when cystine was added, while the raw proteins of the navy and lima beans, similarly supplemented, did not promote growth. These figures are given in table 1.

[3] For the composition of the salt mixture see Osborne and Mendel, Journ. Biol. Chem., 1917, xxxii, 374.

SUMMARY

Raw or cooked adsuki bean meal supplemented with cystine furnished adequate protein and water-soluble vitamine for normal growth. Similar diets without the addition of cystine enabled the albino rats to grow at only one-third to two-thirds of the normal rate. Comparable results were obtained with the isolated adsuki bean globulin.

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THE EPINEPHRIN OUTPUT ESTIMATED BY COLLECTING THE ADRENAL BLOOD WITHOUT OPENING THE ABDOMEN

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Received for publication February 15, 1921

It has been assumed by some writers that when blood from the adrenals is collected in the ordinary way through an abdominal incision, the secretion of epinephrin may be artificially increased by the experimental conditions, and that the rate of output estimated under these conditions may therefore be too high. No experimental evidence in support of this assumption has been brought forward. We have nevertheless judged it worth while to compare the output estimated on blood obtained by the abdominal operation, with that estimated on blood obtained by an extraperitoneal operation. It is possible, without opening the peritoneum, to prepare the left lumbo-adrenal vein in dogs through a lumbar incision, so as to collect the blood flowing from the adrenal without obstruction.

A lumbar incision is made, beginning just below the last rib and extending downward about 2½ to 3 inches. The muscles are either cut or the fibers separated until the peritoneum is seen. With the aid of a gauze sponge the peritoneum is carefully separated downward (and backward) from the adjacent tissues, exposing the adrenal gland with its vein entering the cava, or, as is not infrequently found, the renal vein, especially in dogs. During this procedure it is necessary to exercise caution not to tear the lumbar vein or one of the small veins entering it. A loose ligature is placed around the adrenal vein between the gland and the cava. A cannula is inserted into the lumbar end of the lumbo-adrenal vein, after tying small veins entering it, and the course of the adrenal blood directed through the cannula by occluding the adrenal vein at the cava (or renal vein). This is accomplished by traction on the loose ligature or by clipping the vein, the ligature serving as a guide to the adrenal vein.

As will be seen in the experiments quoted, the outputs determined on the blood collected extraperitoneally were within the usual range of the outputs estimated in etherized dogs with blood collected after

opening the abdomen. Further, when the output was calculated on one and the same animal, first, with adrenal blood collected extraperitoneally and then with adrenal blood collected after the abdomen was opened, no clear difference could be made out, within the limits of error of the method of estimation.

In the first experiment to be cited (dog 355) blood was obtained from the left adrenal through a lumbar incision. The abdomen was then opened and blood collected from both adrenals.

Condensed protocol; dog 355; female; weight 17.85 kgm.

11:25 a.m. to 12:00 m. Under morphine and ether, *via* extraperitoneal route (lumbar incision), inserted cannula into lumbar end of left lumboadrenal vein. By occluding adrenal vein at the junction with the cava, with a small clip, collected blood from the left adrenal.

12:01 p.m. First specimen, 2.75 grams in 30 seconds (5.5 grams per minute).

12:01½ p.m. Second specimen, 10.9 grams in 3 minutes (3.6 grams per minute). Removed clip from left adrenal vein.

12:10 to 12:35 p.m. Abdomen opened, cava pocket completed (coeliac axis and superior mesenteric arteries were tied, in addition to the blood vessels usually tied). Collected blood from both adrenals.

12:35½ p.m. Third specimen, 10.7 grams in 30 seconds (21.4 grams per minute).

12:36 p.m. Fourth specimen, 21.6 grams in one minute. Obtained indifferent (venous) blood specimen. Combined weight of adrenals, 2.3 grams.

The second adrenal specimen, collected from the left adrenal extraperitoneally, caused a much greater inhibition of the intestine segment than the fourth specimen, collected from both adrenals after opening the abdomen, corresponding to the greatly increased flow in the fourth due to the increased blood pressure caused by tying the coeliac axis, superior mesenteric artery and abdominal aorta (fig. 1, observations 48 and 54).

The assay showed that the second specimen was stronger than 1:7,000,000, stronger than 1:5,700,000, somewhat weaker than 1:4,300,000, decidedly weaker than 1:3,500,000 (fig. 1, observations 48 and 50, and other observations not reproduced). The second specimen was assayed at 1:5,000,000, corresponding to an output of 0.0007 mgm. per minute, or 0.00004 mgm. per kilogram per minute for the left adrenal alone. On the assumption that the output is the same for each adrenal, this would give 0.00008 mgm. per kilogram per minute for the two adrenals.

The fourth specimen, collected from both adrenals after opening the abdomen, was found to be decidedly weaker than 1:10,000,000 adrena-

lin, weaker than 1:13,000,000 (confirmed by several sets of observations), weaker than 1:14,300,000, not much different from 1:17,000,000. Taking it at 1:17,000,000, we get 0.0013 mgm. per minute as the output from both adrenals, i.e., 0.00007 mgm. per kilogram of body weight per minute. The output per kilogram of body weight is less than the average, but still within the normal range. We do not know whether the average output per kilogram is the same for large as for small dogs, but the matter is of no importance for our present question.

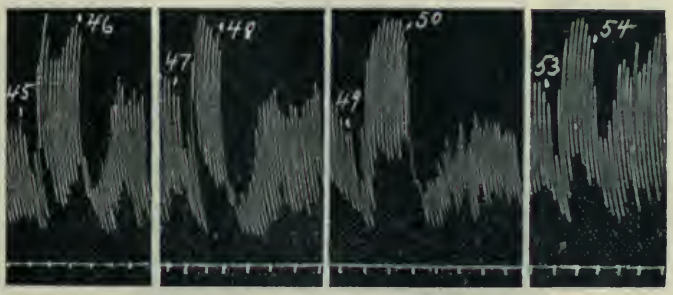

Fig. 1. Intestine tracings. Bloods from dog 355. At *45*, *47*, *49* and *53*, Ringer was replaced by indifferent (jugular) blood and this, at *46*, by indifferent blood to which was added adrenalin to make a concentration of 1:7,000,000; at *48*, by the second adrenal blood specimen; at *50*, by indifferent blood to which was added adrenalin to make a concentration of 1:3,500,000; at *54*, by the fourth adrenal specimen. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). Time trace in half-minutes. Reduced to one-half.

In the next experiment (dog 487) the left lumbo-adrenal vein was permanently tied off near the cava after specimens of the adrenal blood (first, second and third) had been collected. The abdomen was then opened and blood collected from the remaining (right) adrenal in the usual way.

Condensed protocol; dog 487; male; weight, 13.78 kgm.

10:00 to 10:30 a.m. Under ether, *via* extraperitoneal route (lumbar incision), inserted cannula into lumbar end of left lumbo-adrenal vein. By occluding adrenal vein at the junction with the cava, collected blood from left adrenal.

10:31½ a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute).

10:32 a.m. Second specimen, 10.9 grams in 3 minutes (3.6 grams per minute).

10:35 a.m. Third specimen, 11.0 grams in 3 minutes (3.7 grams per minute).

10:40 to 11:03 a.m. Tied left adrenal vein. Abdomen opened and cava pocket completed in usual manner, the coeliac axis and superior mesenteric artery not being tied. Collected blood from right adrenal.
11:04 a.m. Fourth specimen, 5.0 grams in one minute.
11:05 a.m. Fifth specimen, 15.2 grams in 3 minutes (5.1 grams per minute). Obtained indifferent (arterial) blood specimen. Combined weight of adrenals, 1.7 gram.

The second and third adrenal specimens had about the same concentration of epinephrin. They were shown to be much stronger than 1:5,300,000, stronger than 1:2,700,000, decidedly weaker than

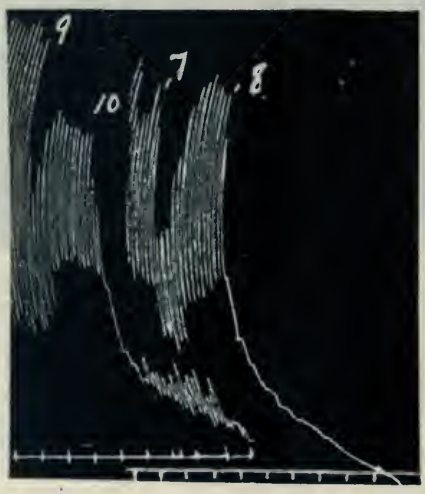

Fig. 2. Intestine tracings. Bloods from dog 487. At *7* and *9* Ringer was replaced by indifferent (arterial) blood and this, at *8*, by indifferent blood to which was added adrenalin to make a concentration of 1:1,325,000; at *10*, by the third adrenal blood specimen (collected from left adrenal by extraperitoneal route). All the bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

1:1,350,000 (fig. 2, confirmed by several other observations). As the reaction when the blood was simply diluted with three volumes of Ringer's solution was too strong for an exact assay, the third specimen was now diluted with one volume of indifferent blood before dilution with Ringer's solution. In this way the third specimen was shown to be somewhat weaker than 1:2,000,000, stronger than 1:2,650,000 (fig. 3, confirmed by other observations not reproduced). It was assayed at 1:2,300,000, corresponding to an output of 0.0016 mgm.

per minute, or 0.00012 mgm. per kilogram of body weight per minute for the left adrenal alone. This is fully equal to the average output for dogs, under the conditions of our experiments (1), when the adrenal blood is collected after opening the abdomen (average 0.00022 mgm. per kilogram per minute for the two adrenals).

The fifth adrenal specimen, collected from the right adrenal after opening the abdomen, had a concentration of epinephrin not very different from that of the second and third specimens, although the blood

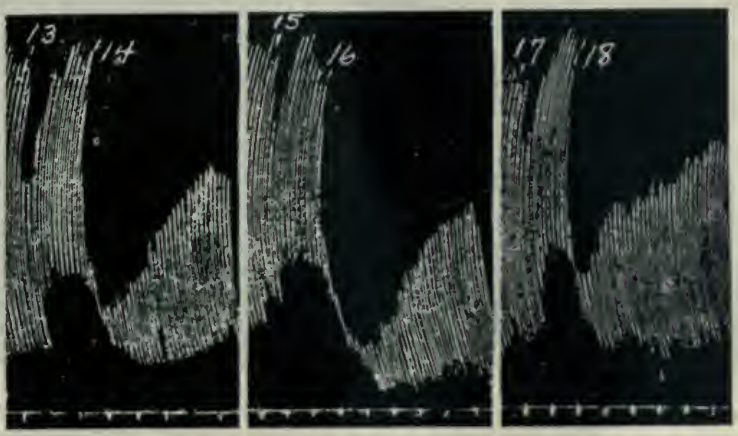

Fig. 3. Intestine tracings. Bloods from dog 487. At *13*, *15* and *17*, Ringer was replaced by indifferent (arterial) blood and this, at *14*, by the third adrenal blood specimen (collected from left adrenal by extraperitoneal route) diluted with one volume of indifferent blood; at *16*, by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at *18*, by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000. All the bloods were diluted with three volumes Ringer (the adrenal blood after adding the indifferent blood, and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

flow was somewhat greater, as the blood pressure had been raised by tying the abdominal aorta when the abdomen was opened. The fifth specimen was shown to be decidedly stronger than 1:2,650,000, weaker than 1:1,325,000 (fig. 4, confirmed by other observations not reproduced). The assay was now continued with the fifth specimen diluted with one volume of ind fferent blood, and the fifth specimen was found to be somewhat stronger than 1:2,650,000, but decidedly weaker than 1:2,000,000 (fig. 5, observations 32 to 36). Qualitatively it was shown

again that there was no great difference in concentration between the third and the fifth specimens (fig. 5, observations 42 and 44), whereas the first (preliminary) specimen was much weaker than the second (fig. 5, observations 38 and 40). This indicates that manipulation in the insertion of the cannula by the lumbar route was not in any way responsible for the output of epinephrin in the second and third speci-

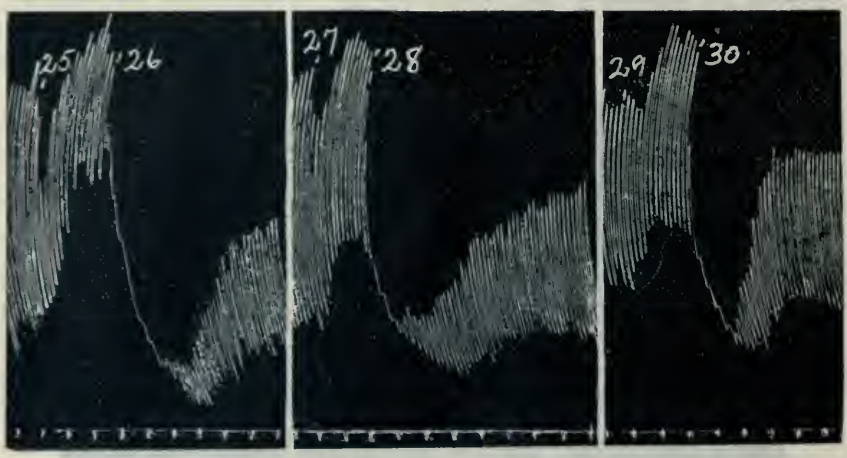

Fig. 4. Intestine tracings. Bloods from dog 487. At *25*, *27* and *29* Ringer was replaced by indifferent (arterial) blood, and this, at *26*, by indifferent blood to which was added adrenalin to make a concentration of 1:1,325,000; at *28*, by the fifth adrenal blood specimen (collected from the right adrenal by "cava pocket"); at *30*, by indifferent blood to which was added adrenalin to make a concentration of 1:2,650,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

mens. The fifth specimen was taken at 1:2,500,000, corresponding to an output of 0.002 mgm. per minute or 0.00014 mgm. per kilogram per minute for the right adrenal, practically the same as the output obtained for the left adrenal with extraperitoneal collection of the blood. It is obvious that the opening of the abdomen could not have sensibly increased the output of epinephrin from the right adrenal.

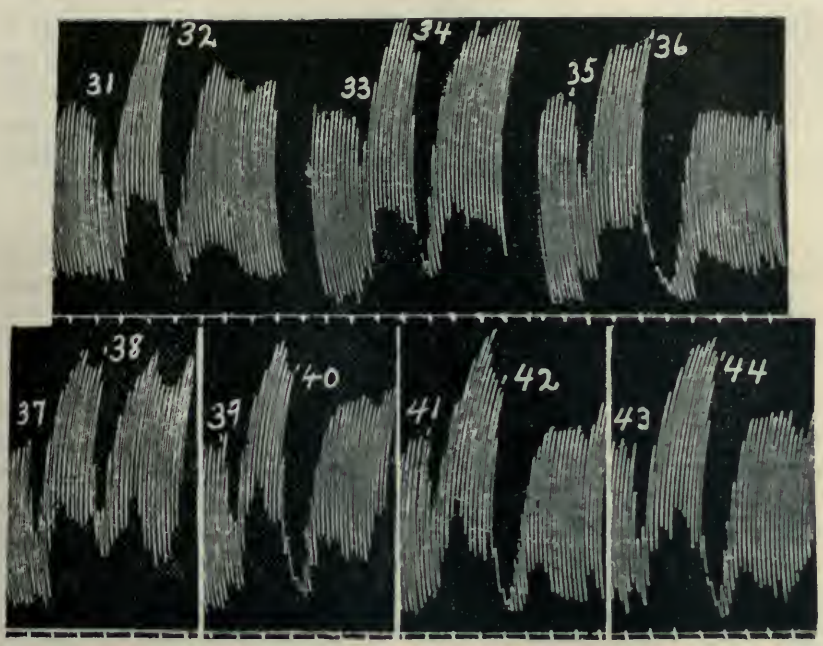

Fig. 5. Intestine tracings. Bloods from dog 487. At *31*, *33*, *35*, *37*, *39*, *41* and *43* Ringer was replaced by indifferent (arterial) blood, and this, at *32*, by the fifth adrenal blood specimen (collected from the right adrenal by "cava pocket"), diluted with one volume indifferent blood; at *34*, by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000; at *36*, by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at *38*, by the first adrenal specimen (collected from left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *40*, by the second adrenal specimen (collected from the left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *42*, by the third adrenal specimen (collected from the left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *44*, by the fifth adrenal specimen diluted with one volume indifferent blood. All the bloods were diluted with three volumes Ringer (the adrenal bloods after adding the indifferent blood, and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

SUMMARY

The rate of epinephrin output is approximately the same whether it is estimated on adrenal vein blood collected without opening the abdomen or after opening the abdomen.

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POST-OPERATIVE DEPLETION OF THE EPINEPHRIN STORE OF THE ADRENALS

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Received for publication February 15, 1921

The progressive diminution in the epinephrin store of the adrenals, which goes on at least for several hours after an operation when the innervation of the glands has not been interfered with, has been studied by us in a previous paper (1). It might be associated solely with the action of the anesthetic since Elliott (2) has shown that general anesthetics, such as ether, chloroform or urethane, cause depletion of the store of an adrenal whose nerves are intact, as compared with that of its previously denervated fellow. Or the depletion might be due to the effects of the trauma as well as to the anesthetic. In either case the depletion could be caused by an increase in the rate of output of epinephrin through the epinephrin-secretory nervous mechanism, without change in the rate of formation of epinephrin, or to interference with the formation of epinephrin, while the liberation went on without change from the innervated gland, or finally to any disturbance of the normal balance between production and output in favor of the latter. That trauma as such, or at least the trauma associated with abdominal operations for the study of the epinephrin output, does not influence the output in an important degree, is indicated by the observations in the immediately preceding paper (3), in which it is shown that the epinephrin output in dogs is, within the limits of error of our experimental methods, the same when estimated upon adrenal blood collected extraperitoneally through a lumbar incision, and upon adrenal blood collected after opening the abdomen. There remains the possibility that the trauma might interfere with the filling up of the epinephrin store. The experiments summarized in table 1 show, however, that when one adrenal is removed under local anesthesia (ethyl chloride), the wound sutured, and the other adrenal removed 5 to 7 hours later, after killing the animal suddenly, there is generally no depletion

of the epinephrin store of the second adrenal. In 9 out of 15 rabbits the store of the second adrenal, as estimated by the method of Folin, Cannon and Denis (4) was precisely equal to that of the first. In one rabbit the store of the second adrenal was a little greater than that of the first. In two of the remaining rabbits (368 and 400) the difference

TABLE 1

Normal rabbits

NUMBER OF ANIMAL	BODY WEIGHT	ADRENAL WEIGHT		ADRENAL WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT	EPINEPHRIN		EPINEPHRIN PER GRAM OF FIRST ADRENAL REMOVED	EPINEPHRIN PER KILOGRAM BODY WEIGHT	RATIO OF EPINEPHRIN IN FIRST TO THAT IN LAST REMOVED ADRENAL	INTERVAL BETWEEN REMOVAL OF ADRENALS
		Left	Right		Left	Right				
	kgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*		*hours*
359	2.68	467	430	334	0.45	0.45	0.96	0.34	1.00	6½
364	1.48	72	70	96	0.10	0.10	1.39	0.13	1.00	6
367	2.09	200	170	177	0.38	0.38	1.90	0.36	1.00	4¾
368	2.06	160	120	136	0.12	0.15	1.25	0.14	1.25	5¾
369	2.45	300	327	256	0.22	0.24	0.73	0.19	0.92	7½
371	2.22	130	116	110	0.15	0.15	1.15	0.14	1.00	6¾
373	2.06	185	170	172	0.16	0.16	0.86	0.16	1.00	7
375	2.64	285	256	206	0.27	0.18	0.95	0.20	1.50	6
397	3.01	241	200	146	0.21	0.15	0.87	0.14	1.40	6½
400	2.43	206	194	164	0.15	0.12	0.70	0.12	1.25	6
401	2.69	228	212	163	0.20	0.20	0.88	0.15	1.00	5¾
402	2.08	250	246	238	0.13	0.13	0.52	0.13	1.00	5¾
403	2.52	169	176	137	0.18	0.18	1.06	0.14	1.00	7
404	2.57	200	188	151	0.22	0.16	1.10	0.17	1.37	6½
405	2.94	236	221	155	0.20	0.20	0.85	0.14	1.00	6¼
Average..	2.39	222	206	176			1.01	0.18	1.11	

Rabbits 368, 369, 371 and 403 were females, the last in early pregnancy. The left adrenal was removed first in all the animals in the table except 368. The operation was extraperitoneal in 359, 368, 369 and 373; in all the others the peritoneum was opened.

between the stores of the first and second adrenals was not greater than may be found in two adrenals removed simultaneously (1). In 3 of the 15 rabbits there seems to have been a genuine, moderate depletion of the second adrenal as compared with the first. The average of the ratios of the epinephrin store of the first to that of the second

adrenal removed for the whole 15 animals is, however, only 1.11:1. Or putting it in another way, the sum of the epinephrin stores in the adrenals first excised is 3.17 mgm., and in the adrenals excised later 2.92 mgm., giving a ratio of 1.08:1.

In one cat, a young female weighing 1.49 kgm., the left adrenal was removed under ethyl chloride anesthesia by an extraperitoneal operation. It weighed 0.16 gm. and contained 0.20 mgm. epinephrin. The cat was killed 6½ hours later and the right adrenal removed. It weighed 0.182 gm. and contained 0.18 mgm. epinephrin, showing no definite post-operative depletion.

So far, then, as these results go, the influence of trauma on the post-operative depletion, appears to be very slight and inconstant.

A second series of observations was made in the same way on 25 rabbits from which the thyroids and the whole, or the greater portion of the parathyroids had been removed. These rabbits were kindly handed over to us by Dr. David Marine, who had used them in work carried on by himself and Dr. O. T. Manley, and desired to sacrifice them for autopsy.

In most of the rabbits the thyroid operation had been performed 7 or 8 weeks before they were given to us. In rabbits 428 and 429 the thyroids and parathyroids were excised 15 weeks, in rabbits 414 and 426 only 5 weeks before we made our observations on the adrenals. In rabbits 480, 481, 482 and 483 the thyroids were removed more than a year before we got them. In all cases most of the right and left lobes (with the superior parathyroids) was excised, also the whole of the right inferior parathyroid and ½ to $\frac{9}{10}$ of the left inferior parathyroid, or sometimes the whole. In rabbit 360 it is noted that all the left inferior parathyroid was removed, and in 389 that ¾ of it was removed and the blood supply of the stump probably interfered with. The majority of the animals received calcium chloride for some time, and tetany was only observed in one or two cases. When calcium chloride was given it was discontinued at least a month before the animals were handed over to us. Thyroid homeo-transplantation under the skin of the abdomen was done in many of the animals, but the grafts were practically always found negative. The last examination of the grafts, under ether, was about a month to 6 weeks before we got the animals, but in rabbits 414, 425, 426, 428 and 429 the last etherization was only 6 to 9 days before they were handed over to us. It is noted that one of the animals (414) had been repeatedly injected with sheep's erythrocytes, the last injection being made one month before the thyroids and the whole of the parathyroids were removed.

Of the 25 rabbits in table 2, 16 gave practical equality of epinephrin store in the two adrenals, or a difference not clearly surpassing the range of the ordinary variation. Three animals are included in this group in which the store of the adrenal last removed was somewhat

TABLE 2

Thyro-parathyroidectomised rabbits

NUMBER OF ANIMAL	BODY WEIGHT	ADRENAL WEIGHT		ADRENAL WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT	EPINEPHRIN		EPINEPHRIN PER GRAM OF ADRENAL FIRST REMOVED	EPINEPHRIN PER KILOGRAM BODY WEIGHT	RATIO OF EPINEPHRIN IN FIRST TO THAT IN LAST REMOVED ADRENAL	INTERVAL BETWEEN REMOVAL OF ADRENALS
		Left	Right		Left	Right				
	kgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*		*hours*
360	2.32	350	384	316	0.58	0.42	1.66	0.50	1.38	6
362	2.08	408	392	385	0.49	0.40	1.20	0.47	1.22	6½
363	2.14	206	170	176	0.08	0.13*	0.76	0.12	1.62	6
365	2.03	261	250	252	0.23	0.27*	1.08	0.27	1.17	5½
366	2.12	200	200	188	0.18	0.18	0.90	0.17	1.00	4¾
370	1.60	204	190	246	0.13	0.09	0.64	0.16	1.44	7
372	1.90	140	190	174	0.13	0.095	0.93	0.14	1.36	7¼
374	2.08	420	400	394	0.32	0.17	0.76	0.38	1.88	6½
389	2.26	992	878	827	1.12	0.76	1.13	0.99	1.47	7
390	2.26	488	436	409	0.72	0.72	1.47	0.64	1.00	6½
391	1.79	280	292	320	0.20	0.13	0.70	0.22	1.54	6¼
396	2.15	280	252	247	0.31	0.18	1.11	0.29	1.72	7¼
398	1.75	435	356	452	0.45	0.24	1.03	0.51	1.87	6¼
412	2.26	247	228	210	0.20	0.18*	0.79	0.17	0.90	6½
413	1.92	258	222	250	0.24	0.26*	1.17	0.27	1.08	6½
414	2.61	326	306	242	0.36	0.36*	1.17	0.28	1.00	6
426	2.32	252	241	212	0.26	0.24	1.03	0.22	1.08	7
427	1.51	186	190	249	0.11	0.06	0.54	0.15	1.83	6½
428	2.47	296	280	233	0.30	0.26	1.01	0.24	1.15	8
429	2.37	291	278	240	0.26	0.22	0.89	0.22	1.18	7½
430	1.07	108	96	190	0.11	0.09	1.02	0.20	1.22	7
480	1.80	198	212	228	0.18	0.15*	0.71	0.18	0.83	6½
481	2.31	290	311	260	0.27	0.24	0.93	0.23	1.12	6½
482	3.70	410	490	243	0.36	0.42	0.88	0.21	0.85	6¼
483	2.57	300	300	233	0.21	0.21	0.70	0.16	1.00	6¼
Average..	2.13	313	302	287			0.97	0.29	1.27	

* Right adrenal first removed; in all the others the left adrenal was first removed. In rabbits 362, 365, 366, 372, 413, 426, 427, 428, 429, 430, 480 and 481 the adrenals were removed without opening the peritoneum; in the rest the peritoneum was opened. Rabbits 360, 362, 363, 365, 366, 370, 426, 427, 430, 480, 481 and 483 were females; the rest males. Rabbits 414 and 426 were castrated one and four weeks respectively before thyro-parathyroidectomy, rabbits 428 and 429 seven weeks after thyroid operation; 482 was also castrated.

greater than that of the gland first removed. In 9 of the animals an undoubted deficiency of epinephrin developed in the second adrenal. The average of the ratios of the weight of epinephrin contained in the first adrenal to that contained in the second for the 25 animals of the series is 1.27:1. Or, if we add together the weights of epinephrin in the glands first excised, we get 7.86 mgm., as compared with 6.42 mgm. in the glands removed at the end of the experiment, giving a ratio of 1.22:1. The variability of the ratio in the individual experiments (standard deviation, calculated as if the quantities were a number of fortuitously differing quantities, = 0.31) is so great that the probable error of the mean (± 0.042) is greater than in the series of normal rabbits (± 0.031) in spite of the larger number of animals. But in neither series are the ratios well distributed about the mean. Although, therefore, the average depletion in table 2 comes out at about 20 per cent, as compared with 10 per cent in table 1, it would be risky to draw from this the conclusion that trauma has a greater effect in inducing post-operative depletion in the epinephrin store in the thyro-parathyroidectomised rabbits. More weight might perhaps be given to the fact, that the number of animals in which an undoubted depletion was observed was twice as great in these as in the normal rabbits, and also to the fact that the degree of depletion, when it was present, was apt to be greater. It might be thought possible that if the general metabolism of the animal was depressed by the loss of the thyroids, the formation of epinephrin might also be slowed, and that this might be accentuated by the operation while the liberation of epinephrin went on. A depletion of the store would then result, and be more or less marked, of course, in different individuals. It is also not beyond the bounds of possibility that changes in the sympathetic nervous system, associated with the removal of the thyroids and the chief part of the parathyroids, might render it easier for post-operative depletion of the epinephrin store to become evident even in the absence of a general anesthetic.

There is another factor, however, which complicates the matter, apart from the relatively small number of experiments. It will be seen from tables 1 and 2 that while the average bodyweight of the normal rabbits was somewhat greater than that of the thyro-parathyroidectomised rabbits, the average weight of the adrenals was more than 50 per cent greater in the latter group. The normal animals had an average of 176 mgm. of adrenal per kilogram of bodyweight and the thyroidectomised rabbits an average of 287 mgm., a difference

of over 60 per cent. Both averages are somewhat too low, as it had not been intended to use the data for any other purpose than comparison of the epinephrin stores of the two adrenals, and the animals were therefore weighed without removal of the gastro-intestinal contents. Considerable error in calculating the relative weight of an organ in terms of bodyweight is introduced if the intestinal contents are included in the bodyweight, especially in an animal like the rabbit where they vary so greatly in amount. There can be no serious error, however, in comparing the average results in tables 1 and 2, because in both series the bodyweights are the gross weights and the errors due to inclusion of the gastro-intestinal contents would, on the average, be about the same in each. The animals were all fed on the same diet.

Gley (5) gives the average total weight of the adrenals in normal rabbits as 0.25 gram, a much smaller weight than in our animals. The weights of the rabbits, so far as he has given them, are on the average about the same as those in table 1. We are unable to account for this discrepancy, but recognize that the variability of the adrenal weight in animals of the same size is considerable. Herring (6) gives the average as 0.20 gram adrenal per kilogram bodyweight for 3 normal rabbits.

The weight of epinephrin per gram of adrenal in the adrenal first removed, and in which little, if any, depletion due to the operation could have occurred, was on the average 1.01 mgm. in the normal and 0.97 mgm. in the thyro-parathyroidectomised rabbits. Putting it in another way, the total epinephrin in the adrenals removed first in table 1 was 3.17 mgm. and the total weight of the adrenals 3.289 grams, i.e., 0.96 mgm. epinephrin per gram of adrenal. The total epinephrin in the glands first excised in table 2 was 7.86 mgm. and the total weight of these glands 7.718 grams, i.e., 1.02 mgm. epinephrin per gram adrenal. In other words, the hypertrophy of the adrenals in the second group has been accompanied by an almost precisely proportional increase, on the average, of epinephrin. The simplest assumption to explain this would be that the medulla had hypertrophied *pari passu* with the cortex. But this does not necessarily follow. It might be that the medullary cells, without increase in number or total mass, were on the average better filled with epinephrin in the hypertrophied glands, either because of the increased blood flow or for other reasons. The percentage of epinephrin in the largest adrenals may be even somewhat greater than the average. Thus, in table 2 there are 8 rabbits, the first removed adrenal of which weighed more than 0.300 gram.

The total weight of the 8 adrenals was 3.809 grams (average 0.476 gram) and the total weight of epinephrin in them 4.40 mgm. (1.15 mgm. epinephrin per gram adrenal). Apart from the fact that when the epinephrin store is large the error in its estimation is less than when it is small, and that the ratio measuring the depletion can therefore be more exactly estimated, it is conceivable that the large and better filled adrenals may more easily part with a portion of the epinephrin store after operation than the small adrenals with a smaller store.

Be this as it may, the possibility should be considered that it is not the loss of the thyroids and parathyroids as such but the adrenal hypertrophy, however produced, with the associated enhancement of the total epinephrin store, which may be responsible for the apparently greater tendency to post-operative depletion, in the absence of a general anesthetic. For instance, if we compare animals with adrenals within the same range of weight (0.2 to 0.3 gram) from tables 1 and 2, it appears that in 9 normal rabbits the average of the ratios representing the epinephrin in the adrenal first removed to that in the adrenal last removed was 1.16. The average weight of the adrenal first excised was 0.238 gram. In 14 thyroidectomised rabbits with adrenals weighing between 0.2 and 0.3 gram, the corresponding average ratio was 1.20 and the average weight of the adrenal first removed 0.254 gram. Here there was practically no difference between the two groups in the tendency to post-operative depletion. It must be pointed out also that in both tables there are animals with large adrenals and correspondingly large loads of epinephrin which show no depletion whatever.

At first glance it might appear that the tendency to post-operative depletion was greater when the peritoneum had been opened than when the first adrenal was removed by an extraperitoneal operation. If trauma, as such, is really an important factor in the depletion, it might be argued that the depletion should be greater and more frequent after the (supposedly) more severe intraperitoneal operation. It so happens, however, what could not have been known till the animals had been operated on, that the average hypertrophy, or at least the average weight of the adrenal in proportion to the bodyweight, was 40 to 50 per cent greater and the epinephrin load correspondingly greater in the animals (in table 2) subjected to the intraperitoneal operation than in the others. The 12 rabbits operated upon through the peritoneum had a total bodyweight of 26.88 kgm. and a total adrenal weight of 8.988 grams (average 0.33 gram adrenal per kilogram of bodyweight). If the proportion is calculated for each animal separately

and the average then taken it is 0.34 gram adrenal per kilogram bodyweight. The 13 animals (in table 2) operated upon extraperitoneally had a total bodyweight of 26.51 kgm. and an adrenal weight of 6.374 grams (average 0.24 gram per kilogram, and the same if calculated in the other way). If, then, there is a greater tendency for the big adrenal to exhibit post-operative depletion, when no general anesthetic is given, this may be the effective factor rather than the difference in the nature of the operations. As already remarked, with groups of animals so small and showing such a degree of variability in the results, it is not possible to come to any very definite conclusion on this point. The absolute amount of epinephrin in the adrenals in the animals in table 2 is considerably greater than in the normal rabbits (0.61 mgm. per animal, as against 0.42 mgm.). In making this calculation the epinephrin store of the adrenal first removed has been doubled, except in the instances in which the load of the adrenal excised last was not less than that of the first adrenal, in which case the loads of the two adrenals were simply added. This procedure is necessary because when the second adrenal has a distinctly smaller store than the first it must be assumed that its original store was depleted after the operation.

When the total epinephrin is calculated as a fraction of the bodyweight there is, of course, a marked preponderance in favor of the animals in table 2 (average 0.29 mgm. epinephrin, as against 0.18 mgm. per kilogram of bodyweight).

The most definite conclusion we feel able to draw from our results is that the hypertrophy of the adrenals, whether it is to be attributed solely to the effects of removal of the thyroids and parathyroids or not, carried with it a corresponding increase in the epinephrin store, so that on the average the weight of epinephrin per unit of weight of the gland was at least as great in the big as in the small glands, while the weight of epinephrin per unit of bodyweight was much increased in the animals with adrenal hypertrophy.

In the literature one occasionally encounters the statement that "hypertrophied" adrenals have a much diminished content of epinephrin (for instance after action of various toxins). But from the context it is usually apparent that what is called hypertrophy is really edema, a condition which, of course, produces a marked increase in the weight of the adrenal far more rapidly than a true hypertrophy can do, and is associated with great loss or even total disappearance of the epinephrin store (1). As regards the effects upon the adrenals of removal of the thyroids alone or with the parathyroids, there is very little agreement.

Rogowitch (7) saw no change in the adrenals of rabbits after "complete" thyroidectomy. The animals showed no symptoms which he could associate

with the operation in the 2 to 6 months for which they survived. It cannot be known how much of the parathyroids was removed. He did not weigh the glands and made observations on very few animals.

R. G. Hoskins (8) quotes a number of other observers who obtained a negative result in rabbits. He found (9) that the adrenals of the offspring of guinea pigs thyroidectomised before conception were hypertrophied on an average by 20 per cent (expressed as percentages of the bodyweight). In one successful experiment on a bitch the puppies had hypertrophy of the adrenals. Thyroidectomy in new-born guinea pigs had no effect on the adrenals, so far as could be ascertained at the end of 15 days.

Pick and Pineles (10) saw hypertrophy of the adrenals in 2 goats on which thyroidectomy had been performed at the age of 6 to 10 weeks.

Biedl (11) observed a slight hypertrophy of the adrenal cortex in dogs after removal of the thyroids.

Gley (5) found great hypertrophy of the adrenals of rabbits after thyro-parathyroidectomy when they lived a long time. He did not make an assay of the epinephrin, but compared the effect on the blood pressure of dogs of injecting known amounts of the extract of the glands with the effect of similar amounts from the glands of normal animals. He concluded that the epinephrin content of the adrenals was not diminished, unless, after a long time, the animals were suffering from the condition which he speaks of as "myxoedema."

Tatum (22) has described hyperplasia of the adrenal medulla with increase of fatty material in the cortex, as a result of complete removal of the thyroid in rabbits 2 to 3 weeks old. The inferior parathyroids were not removed.

Carlson (12) says that "after complete thyroidectomy (young rabbits) we invariably get a hypertrophy of the suprarenals to two or three times their normal size."

Herring (6) states that complete thyroidectomy (with removal of the parathyroids) has little, if any, permanent effect upon the adrenalin content of the suprarenals of the rabbit, although he points out that his experiments were not numerous enough (5 rabbits allowed to live 30 to 73 days after operation). Also there seemed to be no definite effect upon the adrenal weight. None of the animals showed any symptoms.

It has been stated by various observers that feeding with thyroid substance,—R. G. Hoskins (13), Herring (14), E. R. Hoskins (15), Hewitt (16)—or injecting with an ether-soluble material derived from the thyroid—Iscovesco (17)—causes hypertrophy of the adrenals in various animals (guinea pigs, rats, rabbits, cats). Herring also concluded that the epinephrin content of the adrenals and especially the weight of epinephrin in proportion to the bodyweight are increased. Kuriyama (18) maintains that the variations found by Herring both in the adrenal weight and epinephrin content fall within the normal range, and could find no evidence that in the rat there was any special influence of thyroid feeding. In any case, thyroid substance is stated to cause an increase in size in so many different organs, that hypertrophy of the adrenal, associated with thyroid feeding, would be no indication that any special physiological relationship exists between the thyroid and the adrenals, and would certainly be no indication that hypertrophy of the adrenals might not also be associated with thyroidectomy. Many conditions would seem to affect the size of these glands. Thus Guieyesse (19),

Watrin (20) and other observers consider their hypertrophy during pregnancy a normal physiological event. Hatai (21) has asserted that they hypertrophy in female rats, subjected to prolonged muscular exercise daily for several months.

SUMMARY

The question of the relative importance of the anesthetic and the trauma in causing post-operative depletion of the epinephrin store of the adrenals, was tested by removing one adrenal (in rabbits) under local anesthesia (ethyl chloride) and the other after killing the animal after an interval of 5 to 7 hours. In 12 out of 15 rabbits no evidence of depletion was found. In the remaining three there seems to have been a genuine moderate depletion of the second adrenal as compared with the first. In one cat there was no depletion. The post-operative depletion observed in operations under general anesthesia appears, therefore, to be related to the anesthesia in a greater degree than to the trauma.

In another series of 25 rabbits which were handed over to us by Dr. David Marine, and on which thyro-parathyroidectomy had been performed, similar observations were made on the epinephrin store. The average weight of the adrenals in these animals in proportion to the bodyweight was considerably greater than in the normal rabbits. The average weight of epinephrin per unit weight of adrenal was the same in these animals as in the normal series. The store had therefore increased on the average in the same proportion as the gland weight. The average weight of epinephrin per kilogram of bodyweight was markedly greater than in the normal series.

Sixteen of the 25 rabbits showed no evidence of post-operative depletion. In the remaining 9 animals there was an undoubted deficiency in the epinephrin store of the adrenal last removed, as compared with its fellow.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 56 JUNE 1, 1921 No. 2

STUDIES ON THE CEREBROSPINAL FLUID

VII. A Study of the Volume Changes of the Cerebrospinal Fluid after Adrenalin, Pituitrin, Pilocarpine and Atropine

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Received for publication February 7, 1921

In an earlier paper the methods employed in the study of the movements of the cerebrospinal fluid were carefully reviewed and discussed at considerable length (1). It was pointed out that the *outflow method* so commonly employed was unsatisfactory for the following reasons: *1*, The preliminary drainage of the fluid reduced the pressure within the skull, so that new formation might or might not be by the normal mechanism. *2*, Since contained in so complicated a system it obviously is impossible to remove all the fluid present at the beginning of the experiment and thus to differentiate between preformed and newly formed fluid. A part of the fluid flowing from the needle may thus be fluid flowing from a higher to a lower level under the influence of gravity, or of other pressure. *3*, It was impossible by this method to judge the effect of arterial and venous change and the readjustment, for the fluid which had fallen from the tube was lost, and could not reënter the canal if the opportunity presented itself. *4*, It is possible for blood to accumulate in the sinuses, displacing fluid through the needle, thus setting up a pseudo-secretion without any new formation of fluid. *5*, It is difficult, if not impossible, to control the effects of respiratory movements.

The *manometric method* was considered more suitable but open to some criticism: *1*, Pressure conditions are such as to favor the absorption of fluid along the natural channels. Thus the rise in pressure

might mean new formation, while the fall which followed might mean absorption along the normal channels by the usual mechanism. It was shown, however, that the fall occurred just the same with the jugulars ligated as with the jugulars open, and therefore, since the jugular are conceded to be the pathway of absorption, absorption could not have been the cause of the fall. *2*, It must be conceded that some of the fluid of the body can be formed against high, others only against low pressure. It is possible, therefore, that the pressure of formation of the fluid might be so low as to be checked by the pressure found at times in the manometric method of determination. It was with the idea of eliminating this possible criticism that the present work was undertaken.

The method of study employed is open to some of the errors pointed out for the outflow method. It reduces the pressure, it is slow in revealing pressure changes both when pressures are rising and falling, but especially in the latter case it does not differentiate between preformed and newly formed fluid, but *it does show that under the influence of drugs, if the fluid pressure is kept a constant, outflow and inflow are equally a part of the picture* of the action, and thus under the influence of drugs there is absolutely no evidence of new formation of fluid. This then is further proof of our contention that the increased outflow and increased fluid pressure observed are to be explained upon a purely mechanical basis, the result of changes in the arterial or venous pressures or both.

Method. The animal was anesthetized by the intraperitoneal injection of chloretone in oil in dosage sufficient to produce complete anesthesia and immobilization in from 15 to 30 minutes. The animal was then tracheotomized and no further anesthetic was required. Complete immobilization is absolutely necessary in this method because even the slightest voluntary movement is liable to puncture the medulla or cause hemorrhage into the canal, rendering the animal unfit for further study.

The amount of chloretone needed is not sufficient materially to reduce the reactivity of the centers, for the graphs all show typical reactions to the drugs which were subsequently given. Arterial and venous pressures were taken as described in the earlier paper. In most cases we recorded the venous change by direct readings, as well as by a tambour adjusted to the drum. The tambour record is only approximately, the number readings of venous pressure absolutely correct. In the case of the fluid we recorded, instead of pressure, the volume

changes. A short, wide hypodermic needle was attached by means of a short piece of gum tubing to the bottom aperture of a Mariotte bottle having a transverse diameter of 50 mm. and a total capacity of 150 cc. In a bottle of this size the addition of 1 cc. of fluid raises the level by almost exactly 0.5 mm. Thus since the amount of fluid displaced amounts rarely to more than 2 cc., the change in level between the lowest and the highest point will be only about 1 mm., an amount insufficient to produce any appreciable difference in pressure. After the needle was thrust through the atlanto-occipital membrane the bottle was placed on a stand so adjusted that the bottom was level with the midline of the canal, and so inclined that the side to which the tubular aperture was fused was 4 mm. lower than the other. This inclination was established in order to insure that fluid and not air would reënter the canal as long as any was present in the bottle. In this arrangement when the ligament is punctured fluid flows from the canal into the bottom of the bottle until an equilibrium is established, ordinarily about 2 cc. enter. Ten minutes were allowed for the establishment of the equilibrium, then the upper aperture of the bottle was connected by means of air tight tubing to a large-bowled but very delicate tambour covered with moderately heavy rubber dam stretched barely taut across the bowl. Since the bowl was large (5 cm.), the rubber almost slack and the volume change small, the amount of tension exerted by the rubber stretched by the admission of fluid into the bottle is negligible. The writing point of the tambour wrote on the drum above the blood pressure and parallel with the venous pressure.

By this means we were able to measure accurately graphically the volume increase of fluid within the bottle without modifying the pressure, thus permitting the fluid to flow from or into the canal with the minimum of interference. In an experiment in which venous pressure increased and the fluid outflow increased there was room in the bottle for the increase without altering the pressure; when the venous pressure returned to the normal the fluid was available to reënter the canal if the opportunity was offered. In this way we were able to measure *the volume change in the fluid in the canal* plus a reservoir with a total capacity of about 200 cc.; the bottle, rubber tubing and tambour bowl. We standardized the system after each experiment by clamping the tube between the Mariotte bottle and the needle, and injecting known amounts of water into the bottle, and recorded the amount of change in the tambour lever in the usual manner. If there is new formation under the influence of drugs the fluid should flow out into the bottle in amount

equal to the amount formed, then when vascular readjustment takes place the fluid should remain in the bottle for there has been no increase in pressure in the system to force it back into the canal as was the case where the manometer was used. As we see the conditions here *if the fluid failed to return to the canal*, it would not necessarily constitute proof that there had been new formation, because there might be lacking within the canal the force to compel the return of the fluid, but *if the fluid returns, then this method gives the required proof that there has been no new fluid formation*, otherwise there should be no room in the canal for the fluid forced out. As stated before, this method is open to the criticism that the pressure within the canal is reduced.

We did not feel that many experiments need be performed, especially after we found that the results agreed absolutely with those already published secured by the other method. The only difference in results by this method from those already secured by the other method is the constant one obviously to be expected; viz., venous rise and fluid rise will be more or less synchronous because we are dealing with a definite positive force—the rise in venous pressure—in some cases the rise is slow because of the resistance encountered by the fluid in the needle and at other points in the system. The greatest delay, however, comes in the fall in the fluid volume, because it is to be expected that equalization of differences in pressure under the conditions would be slow, because there could be no great negative pressure developed within to suck the fluid back, nor is there any positive force on the outside to drive it back as in the manometer method, where a great difference in the level of fluid on the two sides of the manometer is developed. Thus the readjustment is slow and tedious in some cases—but in others surprisingly rapid.

Adrenalin. The position taken in the earlier paper was that, in agreement with some earlier writers, we were of the opinion that the changes reported in fluid pressure by the manometric, and the increased outflow by the drop method after the injection of adrenalin are due entirely to vascular changes and readjustments within the skull. The results are exactly the same whether the vagi be cut or paralyzed with atropine. With the rise in arterial pressure in the dog injected with adrenalin comes a very pronounced rise in venous pressure. If the fluid is flowing out of the needle inserted into the fourth ventricle there is a marked temporary increased outflow, most marked while venous pressure is rising, but which may persist so long as arterial and venous pressures are higher than normal. This outflow does not

necessarily cease when the pressures cease to rise. This outflow is seen even if the sinus pressure is kept as nearly constant as possible by permitting the sinus to bleed during the action of the drug. If the dural canal is connected with a water manometer the pressure in the fluid manometer closely follows the venous pressure, the only difference being that the fluid pressure continues to rise after arterial and venous pressures have begun to fall and never reaches the same height. These differences have been adequately explained on mechanical grounds.

To support our earlier claims that in the case of adrenalin we are dealing with mechanical changes we present figure 1. This is a single tracing typical of several others made by this method. In others the return to normal was better than in the case presented. As can be seen from the graph, the arterial curve is typical of adrenalin administered to atropinized dogs. The rise is steep, and practically complete in 15 seconds. A slight rise continued for the next 11 seconds but this is due largely to increased strength of the heart resulting in increased oscillation of the mercury column. Thus the true vasomotor effect is complete in 15 seconds. The venous rise is complete in 16 seconds, and begins to fall away immediately. This fall continued slowly but steadily for several minutes. There is no doubt that there was a slight leak in the venous tambour system, but the venous pressure readings on the second line from the bottom are correct. The fluid volume, as can be seen from the graph, increased for 90 seconds, and when the maximum was reached between 2 and 2.5 cc. had entered the bottle from the canal, an amount which raised the level of the fluid in the bottle by not more that 1.25 mm. Following this with the arterial and venous readjustment, this fluid with the exception of about 0.6 cc. returned to the canal. It must be noted that arterial pressure was 10 mm., the venous pressure 38 mm. higher at the end than at the beginning of the observation, a difference sufficient to explain why the 0.6 cc. failed to return to the canal, its place being occupied by the increased amount of blood in the sinuses under the increased pressure. In two other experiments, graphically less satisfactory, the return was perfect in both cases.

Since the conditions in this experiment reproduce the conditions during the period of active outflow in the typical outflow experiment and since it is perfectly clear that the fluid returns to the canal, we conclude that the fluid which escapes from the canal by the outflow method during the pressor phase of adrenalin activity is not newly formed but is preformed fluid mechanically forced out of the canal by increased arterial and venous pressure. Where conditions are absolutely

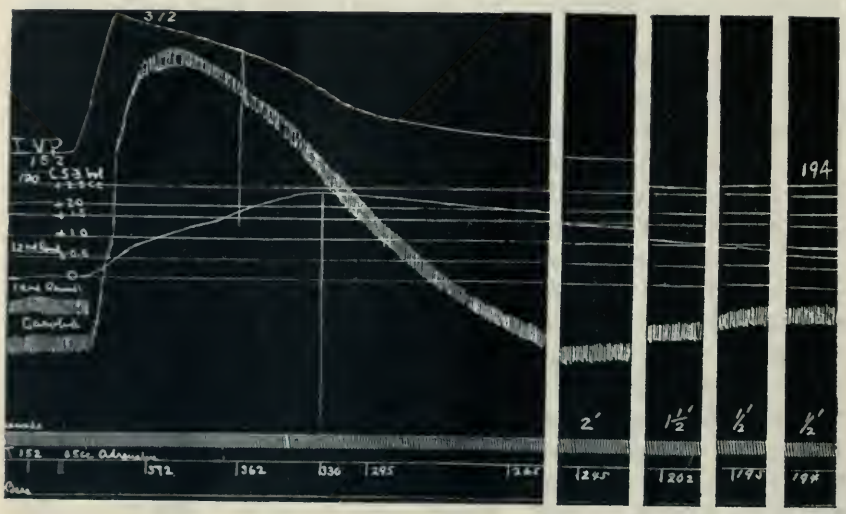

Fig. 1. The effect of adrenalin on venous pressure and fluid volume

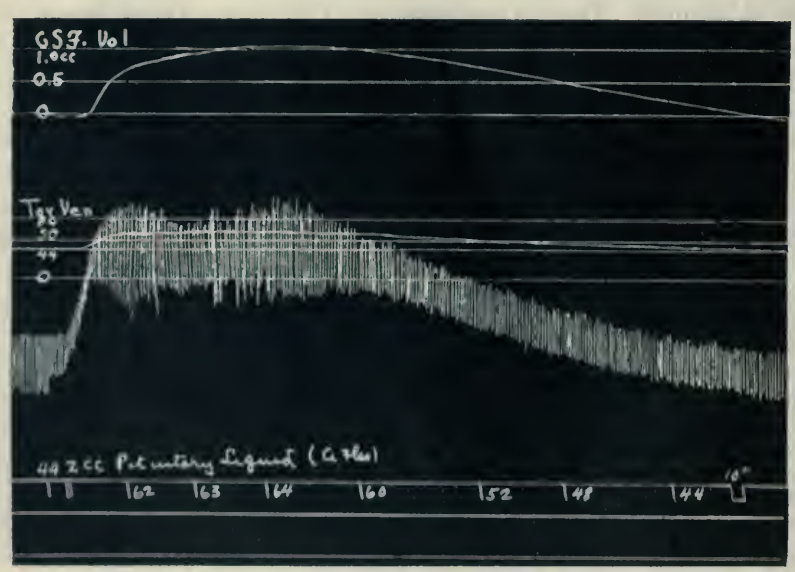

Fig. 2. The effect of the pituitary liquid on venous pressure and fluid volume

satisfactory, outflow and return are equal, and since by this method the pressure is kept under the normal so that the question of too high a pressure for formation cannot enter, as was the case by the manometer method, we conclude that we have conclusive evidence *that there is no new formation* of fluid *during* any phase of *adrenalin action.* The increased outflow, and the increased pressure of the fluid being due only to the alteration of the amount of blood and the changes of the pressure in the arteries and veins within the skull.

Pituitary extract. When the same method of study was applied to extract of the pituitary, equally striking results were obtained. Of four experiments the most satisfactory is shown in figure 2.

In this case the arterial pressure curve is a typical curve for a normal dog; a steep rise with a certain degree of slowing, which persisted after the arterial rise had disappeared, and a slow return of the blood pressure almost to normal after about 20 minutes. The venous pressure rose at first abruptly from 44 to 62, then more slowly to 64, and then fell gradually to 44. The fluid curve was almost exactly synchronous, a very small fraction over 1 cc. of fluid being displaced at the point of greatest arterial and venous pressures, and the return was, as far as venous pressure and fluid volume are concerned, exactly to the starting point at the same time, viz., 8 minutes after the injection. At that time the arterial pressure was 6 mm. lower than normal. It is thus perfectly clear that in this case following the injection of 2 cc. of the commercial extract of pituitary, the rise in arterial and venous pressures forced out 1 cc. of fluid into the bottle; this fluid rapidly made its way back into the canal during the period of readjustment of the vascular condition within the skull. New formation should have been possible in this case for the pressure was never appreciably increased (0.5 mm. of water) during the experiment and the return was not forced by any outside pressure. We therefore have made it clear that: The injection of extract of the pituitary is followed by the mechanical forcing out of the fluid from the canal during the rise in arterial and venous pressure, even if the fluid pressure is kept low this fluid returns to the canal in an amount exactly equal to that forced out during the pressor phase of action of the drug. We conclude: There is no increase in the amount of fluid in the canal during the action of pituitary extract, therefore, *pituiturin does not increase the rate of formation of the cerebrospinal fluid.*

Pilocarpine. As has already been stated, of all drugs having the power of stimulating the secretory mechanism of animals to activity, pilocarpine possesses this action to the highest degree. Thus if a

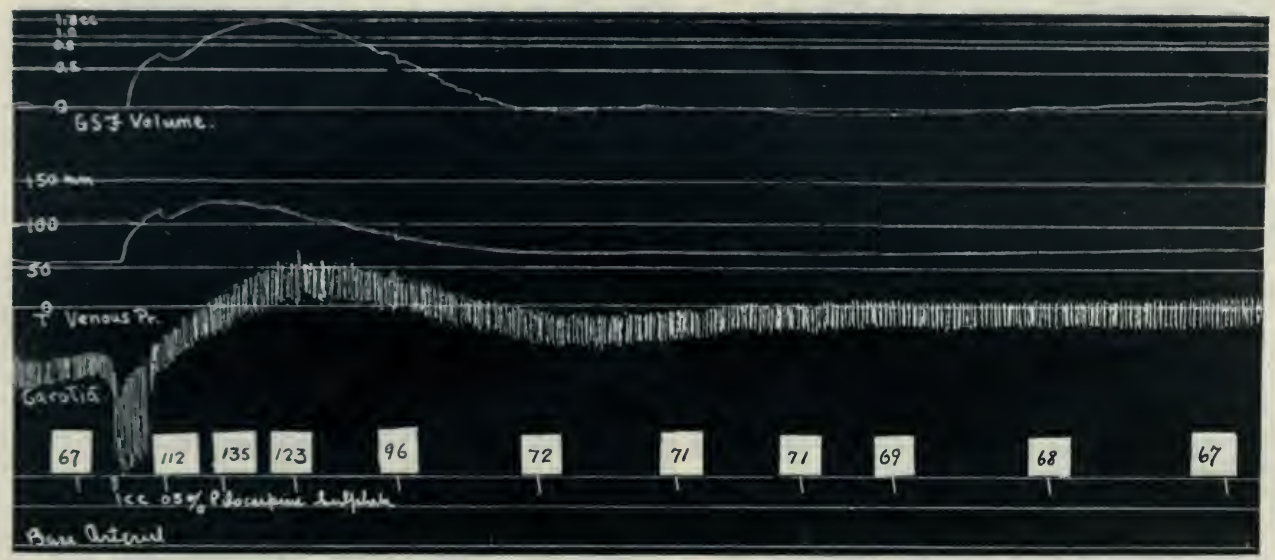

Fig. 3. The effect of pilocarpine sulphate on venous pressure and fluid volume. Note the remarkable similarity between the venous pressure and fluid volume curves.

secretory response on the part of the mechanism forming the fluid is to be expected in any case it is to be expected after pilocarpine. The changes in the venous and arterial pressure are sufficient to explain the changes in fluid pressure and in fluid outflow. In order to cover the ground more fully, we applied this additional method to the study of the action of pilocarpine upon the fluid. As a typical experiment we show figure 3. The time elapsing between the injection and the end of this record is almost exactly 15 minutes. The arterial curve is one typical of a small dose of pilocarpine, with a short period of inhibition and fall in arterial pressure, followed by escape of the beat from the vagus, followed by a rise in the arterial pressure. As can be seen from the graph the venous pressure in the skull rose sharply from the first, fell slightly during the arterial decline, then completed its rise, the maximum coming in 1 minute and 20 seconds, and then slowly fell exactly to normal (shown by the readings). The fluid volume curve is exactly similar, even the irregularities present on the venous curve being reproduced, except that the maximum came 40 seconds after the venous pressure maximum. The total fluid escaping was 1.3 cc. The fluid return also was practically exactly to the level normal before the injection of the drug. Thus it is perfectly obvious that we are dealing in this case with mechanical changes; the rise in venous pressure particularly forcing the fluid out into the bottle, only to permit it to return as the vascular condition of the skull returned to normal. Thus we conclude *small doses of pilocarpine do not increase the rate of formation of the fluid,* the increased outflow or the increased pressure is due to the alteration of arterial and venous pressure within the skull. Larger doses of the drug produce exactly the same effects. The fluid response in this case was small, although the venous pressure rose 69 mm. and the return of fluid to normal was immediate although the venous pressure remained high. When the vagus inhibition was released by atropine there was a temporary rise in venous pressure, a rise in arterial pressure and a prolonged increase in fluid volume forced out into the bottle, an increase persisting throughout the curve. *The fluid eventually all reëntered the canal.* This is undoubtedly the explanation of what happened in the cases reported by Dixon and Halliburton (2) who observed increased outflow following the injection of atropine. In these cases the fluid is forced out by alterations of pressure conditions within the skull, and would return during the period of readjustment. Frequently fluid return to the skull is hastened after atropine.

SUMMARY AND CONCLUSIONS

1. A method is described for measuring and recording graphically the amount of fluid in the skull under slightly less than the normal pressure.

2. Adrenalin does not increase fluid formation, because by this method it can be shown that the fluid flows out of the canal during the pressor stage of the action, but returns in equal amount while vascular readjustment is taking place.

3. In the same way it can be shown that pituiturin does not increase fluid formation.

4. In the same way it can be shown that pilocarpine does not increase fluid formation.

5. Atropine following pilocarpine may—but by no means always—produce increased arterial and venous pressure, accompanied by an increase in the amount of fluid forced into the bottle. The return of this fluid may be slow and tedious but finally takes place. It is believed that this also is a purely mechanical change not accompanied by new formation.

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A STUDY OF THE INFLUENCE OF VARIOUS CIRCULATORY CONDITIONS ON THE REACTION TO THE LOW OXYGEN OF REBREATHING

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Received for publication February 21, 1921

For the purpose of ascertaining whether certain conditions of the circulation may influence the ability for compensating to low oxygen, we selected from 2000 cases ten groups for special study. These selections include men with high and low systolic arterial pressures, high and low diastolic pressures, large and small pulse pressures, rapid and slow pulse rates, and men in whom the systolic pressure rose and others in whom it fell while they were standing.

For each group we have determined the character of the response to low oxygen by calculating the means, with probable errors, for 10 percentages of oxygen during the rebreathing experiment in which 52 liters of air were rebreathed until the subject became inefficient. The individual cases have been listed as to the final oxygen reached and whether the reaction was of the fainting or non-fainting type.

Low and high systolic arterial pressures. Assuming that a low systolic pressure may mean that the nutriment supply to the tissues is inadequate, and that a high systolic pressure, in that it is a measure of vascular and heart strain, might be associated with danger of heart strain during the low oxygen compensation, we selected for special study 26 cases in which the systolic pressure for the reclining posture was 98 mm. Hg. or less and 40 cases in which the reclining pressure was 138 mm. or more.

The compensatory reactions, as determined by calculating means, have been plotted for these two groups in figure 1. Several differences should be noted in the curves. On the whole the reactions are quite alike. However, toward the end of the rebreathing period there occurred in the diastolic pressure a more rapid and greater fall for the high than the low pressure group. The difference is best seen at 8 per

cent oxygen when the diastolic pressure had fallen 15.5 mm. for the high and only 5 mm. for the low systolic pressure group. In the high group the systolic pressure down to 8 per cent oxygen showed a gradual fall, dropping from 157 mm. at 3 minutes to 147 mm., while in the low group the systolic pressure maintained a level, being 114 mm. at 3 minutes and 113 mm. at 8 per cent oxygen. These differences in the systolic and diastolic pressures affect the pulse pressure which at 8 per cent oxygen had increased 16.3 mm. for the high as against 9.2 mm. for the low group.

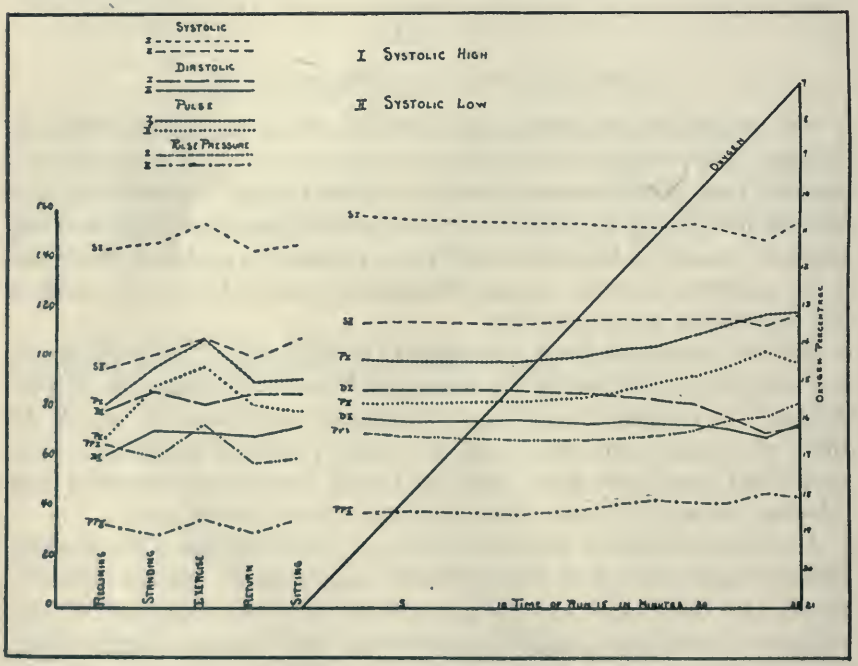

Fig. 1. Composite curves determined by calculating the means for the postural and low oxygen compensatory responses of two groups of men with high and low systolic pressures.

A comparison of the compensatory curves of these two groups with the normal curves of reaction presented in our earlier paper (1) shows that the changes followed the usual course in all factors, except the arterial systolic pressure. In the non-fainting type of response a slight or moderate rise in the systolic pressure occurs during the last 6 or 8 minutes. In neither of these two selected groups was the rise usually present. Seven, 17.5 per cent, of the 40 men with a high systolic pressure showed a terminal rise of from 2 to 10 mm.; while there were 8,

32.5 per cent, of the group of 26 low-pressure cases with a slight systolic rise toward the end. As a rule, therefore, men with a high systolic pressure make the compensation to low oxygen without further increasing the vascular strain by a compensatory rise. To prevent such an increase there occurs a greater vasodilatation than ordinary, as is evidenced by the diastolic pressure fall.

The question of increased strain has been further considered. Prior to the beginning of rebreathing, as the men were seated before the machine, the systolic pressure of the 40 men with a high pressure ranged from 134 to 160 mm. As has been shown only 7 had a final terminal rise, but all showed some degree of psychic rise which was maintained in part by the majority of the cases. So it might be assumed that the pressures met by the heart were extraordinary in some men. In this group the last determination of the systolic pressure, taken just before

TABLE 1

Summary of final oxygen reached

FAILED AT O_2 PER CENT	LOW SYSTOLIC PRESSURE GROUP				HIGH SYSTOLIC PRESSURE GROUP			
	Number of cases	Per cent of cases	Off by psychologist	Off by clinician	Number of cases	Per cent of cases	Off by psychologist	Off by clinician
10.9–10.0	1	3.9		1				
9.9– 9.0	0				3	7.5		3
8.9– 8.0	3	11.5	2	1	4	10.0	1	3
7.9– 7.0	15	57.7	13	2	21	52.5	11	10
6.9– 5.8	7	26.9	7		12	30.0	10	2
Total . .	26	100.0	22	4	40	100.0	22	18

rebreathing was stopped, ranged between 110 and 182 mm. The highest pressures were as follows: 160–169 mm., 3 cases; 170–179 mm., 5 cases and 184 mm., 1 case. The man with a final pressure of 184 mm. went to exactly 6 per cent oxygen. He was 26 years old and his pressure was not above 170 mm. until the last 4 minutes of the test. The oldest man had just past 32 years. The pressures observed ought to be tolerated by young men for short periods of time without injury to the heart and blood vessels.

The percentage of oxygen at which the men of the two groups became inefficient or fainted has been tabulated in table 1. Approximately equal percentages of men with low and high systolic pressures went down to less than 8 per cent of oxygen. Hence it is evident that the height of the systolic pressure does not give an indication of how well a man may compensate to low oxygen.

The members of each group have been classified as to whether they belonged to the fainting or non-fainting type of reactors. Among those having a low systolic pressure 9, or 34.6 per cent, and among those with a high systolic pressure 11, or 27.5 per cent, belonged to the non-fainting type. Here again it is evident that the systolic pressure does not determine the type of reaction that will be made to low oxygen.

There were only 2 men in whom the systolic pressure remained at 100 mm. or less during the entire rebreathing experiment. They compensated to 7.4 and 7.9 per cent of oxygen respectively. The lowest systolic pressure observed in a seated subject was 93 mm. and this man reached 7.4 per cent oxygen. Available evidence indicates that the low systolic pressures here considered, keep the brain as well supplied with metabolism necessities during the conditions of the low oxygen of rebreathing as do higher systolic pressures.

Low and high diastolic pressures. Since it is the diastolic pressure that supplies the force that fills the coronary vessels of the heart, there must be a critical diastolic pressure below which the heart will suffer from a lack of an adequate supply of oxygen and nutriments. In order to determine the possible influence of extremely high and low pressures we have compared a small group of men in whom the reclining posture diastolic pressure ranged between 40 and 56 mm. and another in which it ranged between 90 and 98 mm. There appears to be no outstanding difference in the reactions of the two groups to the low oxygen of rebreathing. Failures from the development of the fainting reaction were more common among the men with a high than with a low diastolic pressure. For the six cases with the lowest diastolic pressure the final oxygen was 8.3, 8.1, 7.6, 7.4, 7.1 and 7.0 per cent respectively. None failed unusually early nor tolerated to an extremely low oxygen. Five of these men were removed by the psychologist at the appearance of complete mental inefficiency. It is evident, therefore, that as a rule the higher brain centers suffered from oxygen-want before the circulation was much affected. Hence it appears that the lowest diastolic pressure found among these aviators did not jeopardize the heart functions in the low oxygen test.

Low and high pulse pressures. Another circulatory measure of the nutritive stream to the tissues is the pulse pressure. Hence it appeared probable that with a low pulse pressure the brain, as well as other tissues, might be more quickly affected by the decrease in the oxygen of the rebreathing experiment than when the pulse pressure is high. We therefore selected 14 cases with the lowest and 19 with the highest pulse

pressure for a comparative study. The low pulse pressures ranged for the reclining posture between 26 and 32 mm., in standing between 20 and 28 mm.; and for the high between 62 and 84 mm. for both postures. By this scheme of selection men with low and high systolic pressures are again brought into contrast. The compensatory curves are therefore quite like those shown in figure 1.

In final oxygen reached by these men there were 37 per cent of the high pulse pressure group who went to 7 per cent or less, while in the low group only 14 per cent went as low. Nevertheless, it is found that when the individual cases are considered, no clear advantage is held by the man with a low or a high pulse pressure. A selection is given below of 5 cases with the lowest and 5 with the highest pulse pressure to illustrate this. The numbers give the pulse pressure at regular intervals throughout the test. The first number in each case is in millimeters of pulse pressure for the subject when seated just before rebreathing was begun. The second gives the pressure during the third minute of rebreathing and registers the psychic increase. The last 4 or 5 numbers bring out the low oxygen effect. The special cases follow:

		Per cent of final oxygen
(1)	25–30–28–30–28–29–30–32–36–30	7.6
(2)	28–46–40–32–31–30–32–36–32	7.9
(3)	31–36–38–36–38–37–33–32–40–52	6.2
(4)	31–42–38–36–40–40–38–44–46–62	7.9
(5)	33–30–32–24–28–30–30–36–34–32	7.3
(1)	73–104–88–80–82–82–92–108–112	8.2
(2)	71–106–104–106–101–100–100–102–122–136	7.4
(3)	71–76–66–70–76–80–72–80–96–88	7.4
(4)	68–80–82–83–80–83–86–90–106–108–112	6.9
(5)	67–70–69–67–62–64–65–64–68–70–74	6.8

There were about equal percentages of men in each group removed by the psychologist because of complete demoralization of voluntary attention and motor coördination. Hence it is evident that no material advantage is gained by either a high or low pulse pressure so far as the maintenance of the brain is concerned.

Rapid and slow rates of heartbeat. On the basis of the belief that a high heart rate indicates a poor physical condition and that physically fit men trained for muscular work show a slow rate, we selected two groups of men to determine what influence the heart rate has on the ability for compensating to low oxygen. There were 123 men with a rapid pulse whose rates were in the reclining posture 87 and above and

in the standing position 108 and above. In the other group with a slow pulse, there were 95 men whose rates in the reclining posture were 60 and less and when standing 75 and less.

The data for the two groups are summarized in the curves of figure 2. That selection on the basis of pulse rate differences does not isolate groups that differ in the vasomotor circulatory factors is well brought out in the arterial pressures for the several postures and during rebreathing. Throughout the entire period of rebreathing, the systolic, diastolic and pulse pressures were about equal and the changes almost identical for the two groups, and quite like those of the average run of cases.

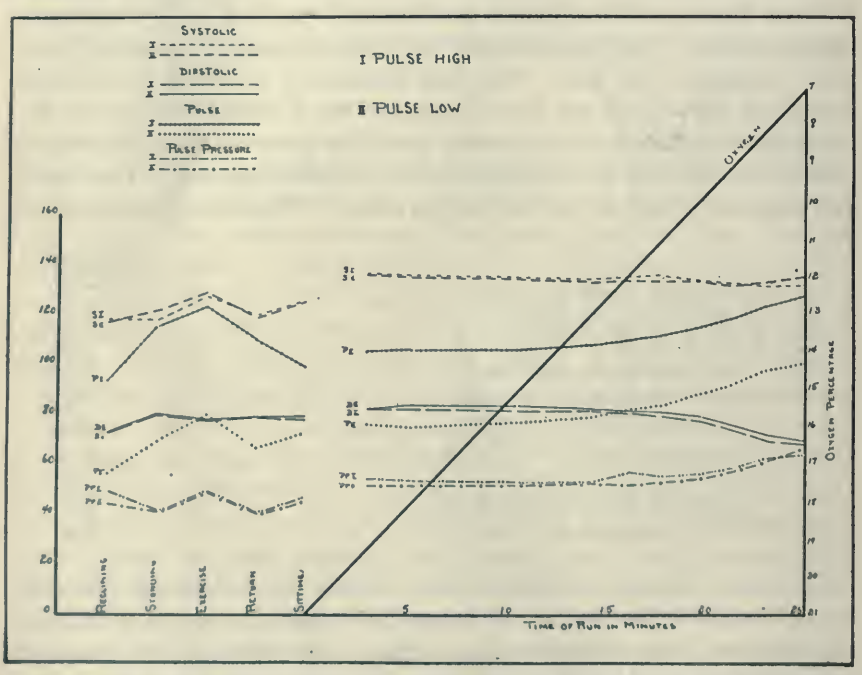

Fig. 2. Composite curves for a group of 123 men with a rapid and 95 men with a slow pulse rate.

The pulse rate curves are also similar, but on different levels. When sitting, just prior to the commencement of rebreathing, the rate for the rapid pulse group was 99 and for the slow 73; at 9, 8 and 7 per cent of oxygen during the rebreathing the increase in the number of beats in the pulse rate was for the former 20, 23 and 28 and for the latter 19,

25 and 28 beats respectively. A more equal degree of response could scarcely be expected on any other basis of choosing cases.

An inquiry to determine how low in oxygen the members of each group went brings out apparently a slight advantage in favor of the rapid pulse group. In this group 82 per cent of the men tolerated to less than 8 per cent oxygen while only 69.5 per cent with a slow pulse went as low. However, the two groups showed equal proportions of those who compensated to less than 7 per cent of oxygen. In the rapid rate group there were 46, or 37.4 per cent, and in the slow pulse rate group 35 cases, or 36.9 per cent, who went below 7 per cent oxygen.

All available evidence supports the conclusion that the initial pulse rate does not give an indication of the manner in which the response will be made nor of the capacity of the individual to respond to the low oxygen of the rebreathing experiment.

Rise and fall of the systolic arterial pressure on standing. It has been assumed that a fall in the systolic arterial pressure with standing gives evidence of physical weakness and unfitness. So we selected, from men who in the recumbent posture had a systolic pressure within the limits of 110 and 122 mm. Hg., 104 men, who showed on standing a compensatory systolic pressure rise of 12 mm. or more; and 117 who showed a fall of 8 mm. or more.

On this basis of selection two groups were obtained in which the mean pulse rates were almost equal and in which there were well-defined arterial blood pressure differences. However, beginning with these differences the two groups maintained them with surprising uniformity throughout the compensations to the low oxygen of rebreathing and showed up about equally well in the percentage of men who went to less than 7 per cent oxygen.

The mean value for the final oxygen percentage was calculated for each of our 10 groups. There was a striking uniformity in which all ranged between 7.2 and 7.7 per cent. The mean value for the final oxygen in a group of 2279 cases was 7.4 per cent. So we find that each of our 10 groups, selected because of a divergence from the average in some one circulatory factor, corresponded very closely to the majority of all men in ability to compensate to the low oxygen of rebreathing. The mean time taken by the 10 groups to reach the final oxygen ranged between 22.3 and 25.2 minutes. This also is close to the mean time found for 2279 cases, which was 24.7 minutes.

The mean increase in the per-minute volume of breathing for each of the groups was also very nearly the same, except in the high systolic

pressure group. This increase as registered for the last minute of rebreathing ranged between 3300 and 3700 cc. For the high systolic pressure group the mean respiratory increase was 4300 cc. per minute. These findings confirm our other data which show that the circulatory factor basis of selection does not separate men that differ in ability to compensate to low oxygen.

SUMMARY

Ten special groups were selected from 2000 cases for a study of the influence of various circulatory factors on the power of compensating to low oxygen. These included high and low systolic pressures, high and low diastolic pressures, large and small pulse pressures, rapid and slow pulse rates and cases of systolic pressure rise and of a fall on standing. All of the groups responded in a similar manner and compensated to equally low percentages of oxygen. None of the conditions studied placed the heart or the nervous system under a handicap greater than is present in average conditions of the several circulatory factors considered.

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THE CORRELATION BETWEEN MOTOR CONTROL AND RIFLE SHOOTING

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Received for publication February 21, 1921

While carrying out a series of student experiments with the steadiness test as described by Whipple,[1] it occurred to one of us (D) that involuntary movements or tremors probably play an important, if not a predominant part in rifle shooting. To test the truth of this hypothesis we carried out a short series of preliminary experiments with a group of five soldiers,[2] two sharpshooters (revolver) and three others who had failed to qualify as marksmen. These preliminary experiments encouraged us to carry out more extensive trials with large numbers of men and improved apparatus. With the coöperation of the office of the Surgeon General, we were able to test seventy-three men of known marksmanship at Camp Meade. In calculating the coefficient of correlation between rifle shooting ability and steadiness we used the rank difference method. This gave us a positive correlation of 0.61. Since this correlation is well within the significant and practically useful region, it may prove to be of value in selecting *a*, men physiologically unfitted to become rifle shots; and *b*, men who show an unusual degree of motor control and are therefore preferred material for intensive range training. The advantages of improving motor control and therefore shooting ability by practice with the steadiness apparatus rather than with firearms will be discussed further on.

Apparatus. Our preliminary experiments were carried out according to Whipple's directions (pp. 156–157), but we soon found several serious practical objections to this method. The Whipple apparatus consists of a brass plate set at an angle of 45° and pierced with two series of holes whose diameters, in sixty-fourths of an inch, are 32, 20,

[1] Whipple, G. M., 1914, Manual of mental and physical tests, Baltimore, Warwick & York, Inc.

[2] We are indebted to Col. O. G. Brown for placing these men at our disposal.

16 and 13 in the top row and 11, 10, 9,8 and 7 in the bottom row. A metallic needle (size not stated) with a flexible connection, is wired in series with the plate, two dry cells and a telegraph sounder or buzzer, in such a way that contact between the needle and plate will actuate the sounder. A writing lever attached to the armature of the sounder records contacts on a kymograph.

The subject is instructed to hold the needle within the largest hole and to maintain this position, so far as possible, without touching the brass plate during the 15 seconds allowed for the trial. The click of the sounder serves as a warning for him that the needle is making contact with the plate. This precaution is particularly necessary in the case of the smaller holes.

The chief objections to this method may be summarized as follows: *1*, The arrangement of the holes in two rows introduces a disproportionately large error in passing from the fourth hole at the end of the top row to the fifth at the beginning of the second row;[3] *2*, when the test is given in a standing position, there is no simple way to adjust the instrument to the varying heights of different subjects; *3*, the counting of the number of contacts on the kymograph is a laborious and time-consuming process, and, in the case of the smaller holes, the *duration* of a single contact introduces a complicating factor.

To overcome these objections to the Whipple method, we used an apparatus essentially like one devised by Dr. Knight Dunlap. Our instrument consisted of two circular brass plates, six inches in diameter, mounted, one-eighth of an inch apart, on the end of a half-inch brass rod ten inches long. The test holes were drilled through the outer plate which could be revolved so as to bring every hole into the same relative position in front of the subject and directly over a thirteen-sixteenths inch hole in the second plate. After dividing the plate into sixteen equal sectors, fifteen holes were drilled five-eighths of an inch from the edge of the plate as follows: 32, 28, 24, 22, 20, 18, 16, 14, 13, 12, 11, 10, 9, 8 and 7 sixty-fourths of an inch respectively. The second plate, containing only the $\frac{13}{16}$ inch hole, covered all the holes in the outer plate except the one into which the needle was inserted. The subject's attention could therefore be concentrated more easily on the test hole.

By means of clamps and a heavy vertical stand, the brass plates were adjusted to suit the heights of different subjects. The plates

[3] Personal communication from Dr. Knight Dunlap.

were set at an angle of 45° and, with the help of a horizontal rod, the top edge of the plate was always placed on a level with the subject's right shoulder. Preliminary trials had shown this adjustment to be satisfactory and quite uniform. Our stylus was made of a 6½-inch piece of number 39 B. and S. stub steel stock (diameter 0.0995 inch) mounted in a light, cylindrical, wooden handle, 3½ inches long and ½ inch in diameter. The brass plate, stylus, two dry cells and buzzer and a double telephone receiver were all wired in series in such a way that the stylus in making contact with the brass plate actuated both the buzzer and the telephone. The buzzer served to warn the subject of prolonged contact in the smaller holes. The brief contacts which failed to sound the buzzer were easily picked up by the operator through the telephone. In place of the kymograph recording device, we used a Veeder mechanical counter and the operator pressed the counter each time the telephone clicked. This simplified method of recording contacts saved an enormous amount of time.

Experimental procedure. The experiments at Camp Meade were carried out in the hospital laboratory.[4] The apparatus was set up on a solid table in such a way that the subject stood with his back to the window; but the plate and stylus were well illuminated. One operator managed the telephone, counter and stop-watch, while the other operator instructed the subjects, adjusted the plates to the proper heights, recorded the names of the subjects on special cards and controlled any tendency on the part of the subjects to withdraw the stylus or to brace the elbow against the body. General instructions were reduced to a minimum by having six men in the laboratory at the beginning of the experiment and, as each man finished his trial, a new man entered and took his place. The test itself is so simple that the men readily understood what was expected of them by watching the five men ahead go through the experiment. As each man took his place before the apparatus, he was shown the significance of the buzzer and he was also given a brief preliminary trial, usually in hole number 16. This was intended as a "shock absorber"[5] and generally served to reassure the subject. In conducting the tests the subject was always allowed 3 or 4 seconds for taking the position in each hole, since a certain amount of movement

[4] We are indebted to the military authorities of the Third Corps Area and at Camp Meade for their generous coöperation in this research.

[5] For this and other excellent practical suggestions regarding the giving of psychological tests see Link, H. C., 1919, Employment psychology, Macmillan Co.

appears when the needle is first inserted that is almost immediately checked by the subject's own control. The time for each trial was 20 seconds. We found no difficulty in giving the test at the rate of twenty men per hour.

Tests made with two groups of students during the past 2 years had shown that hole number 13 is a satisfactory starting point. In the majority of cases each man was therefore tested in holes 13, 12, 11 and 10. When the subject made a total of less than 50 contacts in these four holes he was also tested in number 9. Preliminary experience had shown that when the total number of contacts for any series of holes is greater than about 50 it is generally useless to make observations in the next smaller hole, since the contacts then tend to become so frequent that an accurate count is impossible. We therefore adopted this principle with all subjects. In six cases the subject could not get beyond hole number 11; and the most unsteady man, who was likewise the poorest marksman, made 88 contacts in hole number 13. (See table 3.) It is of interest that this individual was the only subject of more than one hundred tested who made more than 50 contacts in hole 13. This fact seems completely to justify our selection of hole 13 as a practical starting point for the test.

An analysis of rifle shooting. Before discussing our method of scoring the steadiness records and their correlation with marksmanship, a brief analysis of the physiological and psychological aspects of rifle shooting seems in order. Successful rifle shooting requires: *a*, vision sufficiently good to see the sights and target clearly and without blurring; *b*, a high degree of motor control (eye and hand coördination) to enable the marksman to hold the target, the front and the rear sights in alignment; *c*, a slow, steady pressure, not a jerk, on the trigger, the so-called "trigger-squeeze," applied at the moment of sight and target alignment; and *d*, a complete indifference to the sound of the shot, that is, an absence of so-called "gun-shyness" or "flinching." Such technical points as sight elevation and judgment in the matter of cross wind currents need not concern us in this discussion.

Of these four factors the first, vision, can in most cases be corrected with proper glasses. The trigger-squeeze is a matter of practice and presents no insurmountable physiological or psychological obstacle. A "gun-shy" individual, whether man or dog, is generally considered hopeless, since the response is ordinarily quite beyond voluntary control. Probably, in the case of man, a study and exposure of the origin of the gun-shyness would remove the difficulty far more rapidly than

attempting to accustom the individual to the sound of gunfire. As a practical problem, however, gun-shy individuals are relatively rare. In our study we found a single man who made an excellent steadiness record but failed to qualify as a marksman. (See number 66 in table 3, column 1. This individual has the largest D^2 value also.) We suspect this man of being gun-shy but we have no positive evidence for our suspicion.

There remains the factor of motor control. Although an individual with an oscillating gun barrel is at an obvious disadvantage on the rifle range, neither the degree of involuntary tremors nor the control of such tremors has ever received systematic study in the case of marksmen. In our opinion, involuntary movements constitute an important and easily measured barrier to successful marksmanship. It must remain for future investigations to show whether training of involuntary movements by the use of a steadiness apparatus will improve rifle shooting. The practical advantages of preparing men to become marksmen by laboratory training rather than by range practice are too obvious to require further comment.

The rating of marksmanship. Rifle shots in the Infantry of the United States Army are divided into six groups, as shown in the following table.

TABLE 1

GRADE	RIFLE FIRING POINTS	NUMBER OF MEN TESTED
1. Expert rifleman	253	3
2. Sharpshooter	238	17
3. Marksman	202	14
4. First-class man	177	12
5. Second-class man	152	10
6. Unqualified	Less than 152	17

Although all men in the first five grades must attain a high degree of accuracy in estimating distances, the main criterion for ranking rifle shots is actual range score. In our table 3 we have therefore ranked our seventy-three subjects on the basis of their best range scores, made under uniform and carefully controlled conditions. The exact details of distances, number of shots fired, method of scoring targets, etc., are irrelevant to our discussion but may be found in the Small Arms Firing Manual for 1913, issued by the War Department.

Method of scoring the steadiness test. In our laboratory experience with student subjects, we found that the steadiest individuals could hold

the stylus for 20 seconds in holes 13, 12 and 11 without a single contact. As an empirical basis for comparison, we therefore counted all contacts in hole number 10 as single units, but penalized contacts in 11, 12 and 13 by counting single contacts as 5, 10 and 20 units respectively. The

TABLE 2

Method of scoring the steadiness records

HOLE NOS.	CONTACTS		UNITS		SCORE
		Subject 1			
13	0	×	20.0	=	0
12	0	×	10.0	=	0
11	2	×	5.0	=	10
10	3	×	1.0	=	3
9	14	×	0.5	=	7
Total.....	19				$\frac{20}{5}=4$
		Subject 19			
13	2	×	20.0	=	40
12	3	×	10.0	=	30
11	10	×	5.0	=	50
10	22	×	1.0	=	22
9	38	×	0.5	=	19
Total.....	75				$\frac{161}{5}=32.2$
		Subject 47			
13	4	×	20.0	=	80
12	11	×	10.0	=	110
11	19	×	5.0	=	95
10	32	×	1.0	=	32
9					
Total.....	66				$\frac{317}{4}=79.2$

test was so difficult in hole number 9 that in this case two contacts were counted as one unit. The total number of units was then added and divided by the number of holes tested (3, 4 or 5) which gave a steadiness score for a comparative rating. The exact method of scoring with three records taken from our observations is shown in table 2.

TABLE 3

Correlation of marksmanship and steadiness by rank differences

MARKSMANSHIP		STEADINESS		D^2	MARKSMANSHIP		STEADINESS		D^2
Rank	Score	Rank	Score		Rank	Score	Rank	Score	
1.0	266	1	4.0	0.00	38.0	196	34	55.5	16.00
2.0	264	16	27.9	196.00	39.0	193	59	140.7	400.00
3.0	253	25	39.2	484.00	40.0	190	10.5	16.0	870.25
4.0	248	14	25.5	100.00	41.0	189	43	66.5	4.00
5.0	247	5	13.6	0.00	42.0	188	58	135.2	256.00
6.0	244	38	60.5	1024.00	43.0	186	50	88.2	49.00
7.0	242	37	58.6	900.00	44.0	182	66	195.0	484.00
8.0	241	8	15.1	0.00	45.0	179	30	44.5	225.00
9.0	240	60	143.5	2601.00	46.0	178	4	13.1	1764.00
11.0	239	19	32.2	64.00	47.0	176	56	118.2	81.00
11.0	239	12	17.3	1.00	48.0	173	54	108.0	36.00
11.0	239	36	58.4	625.00	49.0	171	31	49.2	324.00
16.5	238	6	14.4	110.25	50.0	169	63	164.0	169.00
16.5	238	21	34.2	20.25	51.0	168	42	66.2	81.00
16.5	238	13	18.3	12.25	52.0	163	62	153.2	100.00
16.5	238	39	61.7	506.25	53.0	158	67	240.0	196.00
16.5	238	15	27.5	2.25	54.0	156	46	76.5	64.00
16.5	238	22	36.5	30.25	55.0	154	35	57.5	400.00
16.5	238	41	65.2	600.25	56.0	153	55	111.5	1.00
16.5	238	2	7.9	210.25	57.0	145	68	270.2	121.00
21.0	230	49	83.2	784.00	58.0	140	51	94.7	49.00
22.0	229	29	44.4	49.00	59.0	137	64	164.5	25.00
23.5	225	32	50.2	72.25	60.0	135	40	64.8	400.00
23.5	225	28	41.5	20.25	61.0	133	17	30.5	1936.00
25.0	218	26	40.2	1.00	62.0	124	71	335.0	121.00
26.0	215	24	39.0	4.00	63.0	122	53	107.7	100.00
27.5	213	23	38.0	20.25	64.0	114	70	316.6	36.00
27.5	213	44	71.7	272.25	65.0	112	33	51.5	1024.00
29.5	209	9	15.6	420.25	66.0	100	10.5	16.0	3080.25
29.5	209	48	79.5	342.25	67.0	90	72	580.0	25.00
31.0	205	27	40.5	16.00	68.0	76	47	79.2	441.00
32.5	204	18	31.6	210.25	69.0	75	69	295.0	0.00
32.5	204	7	14.6	650.25	70.0	67	57	125.5	169.00
34.0	203	52	100.7	324.00	71.0	61	65	186.0	36.00
35.0	199	45	72.7	100.00	72.0	44	61	149.5	121.00
36.5	197	3	11.9	1122.25	73.0	26	73	1280.0	0.00
36.5	197	20	33.2	272.25					

$\Sigma D^2 = 25372.00$

For 73 cases $\rho = +0.61$

The coefficient of correlation between rifle shooting and steadiness. We were interested in determining the degree to which great steadiness and expert shooting ability tend to occur in the same individual. We therefore calculated our coefficient of correlation between rifle shooting and steadiness by the rank difference method.[6]

The formula:

$$\rho = 1 - \frac{6 \Sigma D^2}{n (n^2 - 1)}$$

gives us a value for $\rho = +0.61$. In addition to each man's rank, we have given the actual rifle and steadiness scores in our table 3.

Where several men had the same score as the three men who made 239 on the rifle range (number 11, table 3) they were all given the same rank (11) and in this instance rank numbers 10 and 12 were not assigned. This is the customary statistical procedure. Although the high positive value of the coefficient of correlation is well within the significant range, it must not be imagined that a single expert rifle shot will inevitably make a high steadiness score. Predictive tests are useful as short cuts in grouping large numbers of individuals. They are incomparably more efficient, provided the tests themselves have been properly tested, than the methods of trial and error and learning by experience.

CONCLUSIONS

From the foregoing experiments it is clear that steadiness is definitely associated ($\rho = +0.61$) with marksmanship in the case of men who have had range experience. The practical value of a test of this sort lies in its use as a predictive means of selecting probable expert rifle material and eliminating men who are physiologically unfitted to qualify as marksmen. Since the steadiness test requires only about 3 minutes per man, it would be far more efficient as regards time, cost of munitions and the nerves of men and officers to select material for elimination or range development by this test rather than by the slow and costly method of giving every man a try-out with the rifle. In our experiments we have, however, only tested the test on men of known ability. It remains for future work to show how extensively this test may be used with raw recruits. Range practice may increase a man's steadiness and, conversely, steadiness practice may improve a man's range score. Further experiments alone will tell.

[6] The tables published by the Scott Co., Philadelphia, in the Journal of Applied Psychology, iv, 115, greatly facilitate the computation of the coefficient of correlation by the rank difference method.

A NOTE ON THE RÔLE OF THE INTRINSIC PLEXUSES IN DETERMINING THE EFFECTS ON GASTRIC MOTILITY OF VAGUS STIMULATION

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Received for publication February 23, 1921

In a previous report (1) attention was called to the fact that prolonged tonic contraction of the muscle of the turtle's stomach cannot be induced by electrical stimulation of the vagus nerve. It was also pointed out that by cutting the nerves and allowing the animals to survive for one to two days in crucial experiments no evidence is obtained of tonic stimulating action, but on the contrary hypertonic muscular activity frequently follows destruction of the medulla or cutting of both vagi, thus indicating a tonic inhibitory action of the medullary centers.

It is however a fact that a tetanizing induced current applied to the vagus nerve will cause a generalized contraction of the stomach with or without peristaltic waves. The peculiar fact that this contraction soon disappears although stimulation of the nerve be continued, suggested a study of the rôle of the intrinsic plexuses in relation to vagus stimulation. For this we have contrasted the effects of vagus stimulation on gastric activity with that of direct stimulation of gastric musculature.

Methods. To record the effects of vagus stimulation the turtles were decerebrated, the medulla oblongata being left intact, and a rubber balloon was put into the stomach. This was connected with a water manometer for graphic tracing. In addition to this an opening was made in the back of the shell on the left side to expose the stomach to direct observation. After such an opening the animal can still breathe through the remaining lung and asphyxia is avoided. For direct stimulation of the stomach platinum electrodes were placed directly on the stomach or a strip of gastric muscle was suspended in oxygenated Ringer's solution.

In the work on the dog a gastric fistula was made and after the wound was healed the animal was decerebrated according to Sherring-

ton's method. Graphic tracings were made 6 to 24 hours later, and the vagus stimulated without the necessity of anesthesia.

Results: 1. If the vagus nerve be stimulated with tetanizing induced shocks for a long time (15 minutes or more) the stomach at first contracts strongly and then relaxes at the same rate as follows a spontaneous contraction, although the stimulation is being continued. After a time a second weaker contraction may follow but the stomach soon relaxes and remains quiescent although the stimulation be continued. A sudden increase in intensity of stimulation at this stage may be followed by a contraction and then the stomach relaxes in spite of the

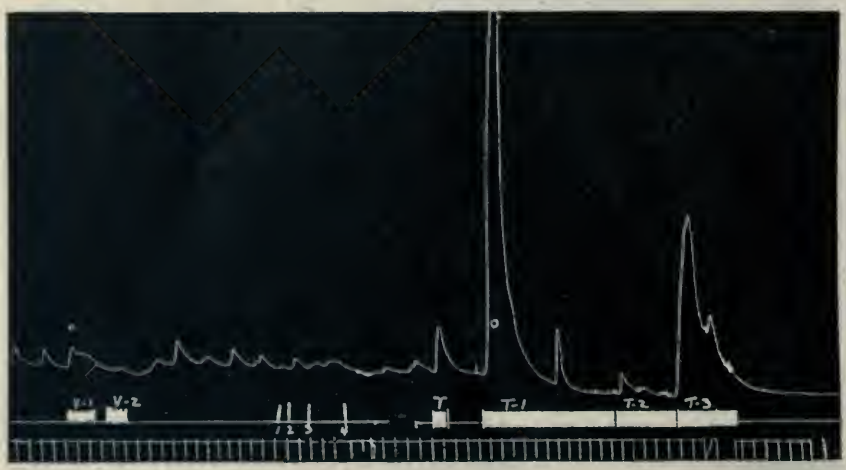

Fig. 1. Gastric contractions of decerebrate turtle. *V–1*, Passage of voltaic current through peripheral end of vagus nerve (6 volts); *V–2*, repeated with twelve volts; *1, 2, 3, 4*, stimulation of vagus nerve with voltaic current by single closing and breaking voltaic current; *T*, weak tetanizing induced shocks applied to the vagus; *T–1*, medium tetanizing shocks to vagus; *T–2*, very strong tetanizing current to vagus; *T–3*, tetanizing current directly to the stomach wall.

continued stimulation (fig. 1). The continuous application of the induced shocks to the vagus does not therefore lead to long-continued increase of muscle tone. On the contrary, after strong contractions the tonus is for a long time lower than that preceding the stimulation. This same fact has been noted in the dog (fig. 2). It seems to be a general rule that maximum contractions induced by vagus stimulation leave the stomach in a state of temporary depression.

2. Continuous passage of galvanic current through the vagus nerve gives no evidence of stimulating effects but sometimes there is a slight inhibitory effect. A series of voltaic stimuli given at the rate of six per second gave the same effects as induced shocks.

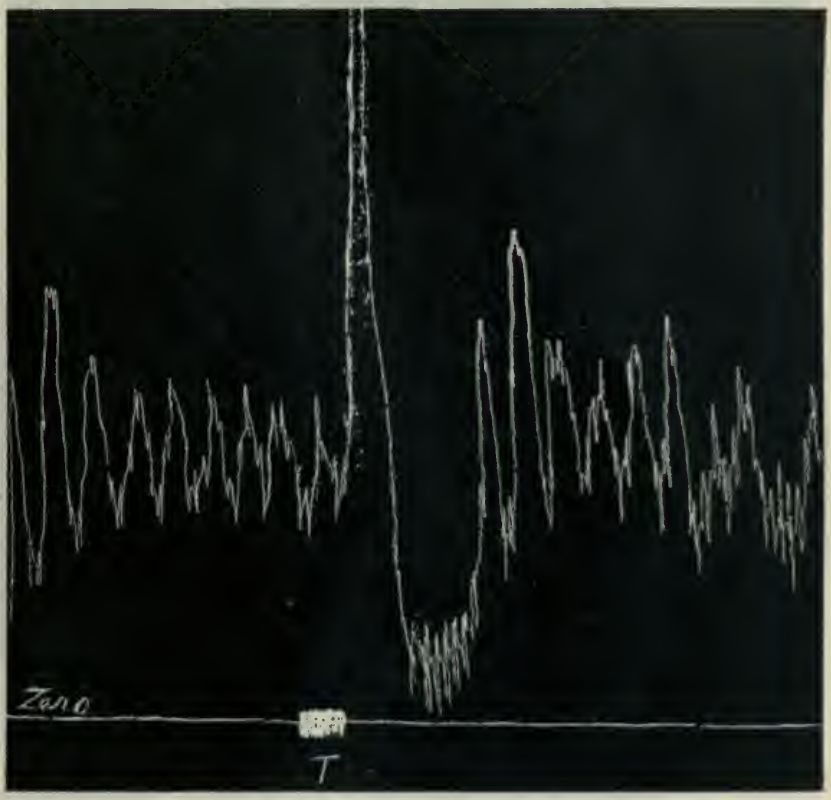

Fig. 2. Weak hunger contractions of decerebrate dog. Induced shocks on peripheral end of vagus. Contraction followed by inhibition.

3. Number of stimuli: A single induced shock, howsoever intense, applied to the vagus nerve, has never in our observation caused a gastric contraction.

4. Rate of stimulation: Single induced shocks at the rate of one every 2 seconds or slower leads to inhibition of gastric tone in the turtle. Single induced shocks at the rate of six per second or faster, lead to contraction. Mechanical stimulation such as handling the nerve or

traction on the nerve leads to inhibition in both turtle and dog (fig. 3). Weak stimulation or a slow rate of stimulation favors inhibition.

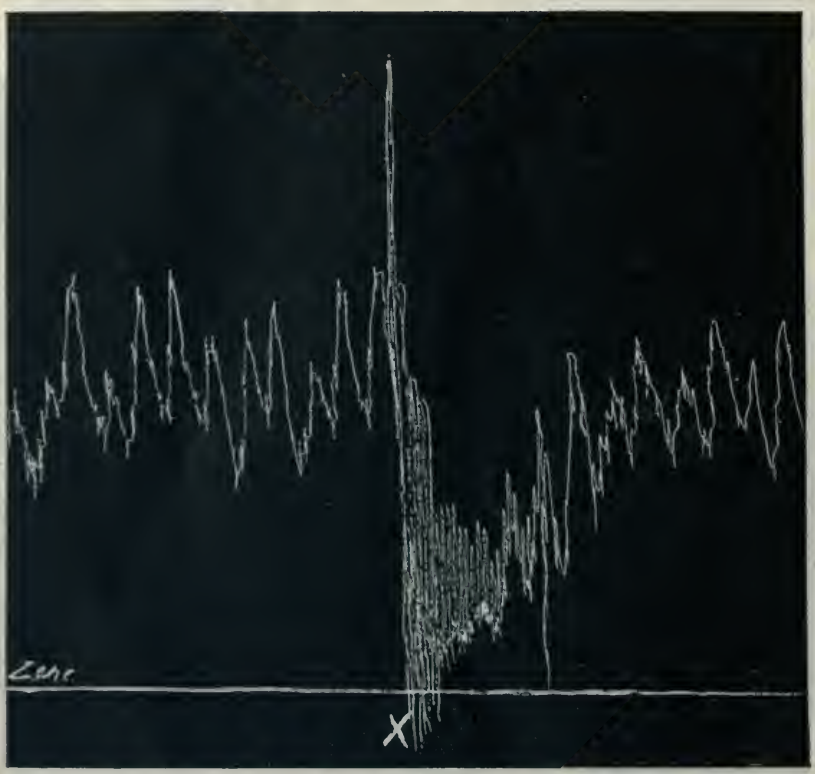

Fig. 3. Hunger contractions of decerebrate dog. *X*, Mechanical stimulation of peripheral end of vagus by traction on ligature.

5. Intensity of stimulation: The greater the intensity of the induced current, the less is the minimum number of induced shocks required to cause gastric contractions.

INTENSITY OF CURRENT	RATE OF STIMULATION	NUMBER OF SHOCKS	RESPONSE
9	6 per second	19	Minimum contraction
8	6 per second	15	Minimum contraction
7	6 per second	10	Minimum contraction
6	6 per second	5	Minimum contraction

Intensity measured on the arbitrary scale of the Harvard inductorium. Intensity "9" was barely susceptible to the tongue and "6" was painful to the tongue.

6. Influence of asphyxia: Prolonged asphyxia (12 to 48 hours) abolished the response of gastric contraction to vagus stimulation. Although when this stage was reached stimulation of the gastric musculature was followed by contraction.

7. Influence of temperature: If the temperature of the turtle is lowered to about 10°C., motor responses to vagus stimulation cease. Increasing the body temperature of the turtle up to 25°C. leads to increased vigor of gastric contraction after vagus stimulation. This temperature effect may of course be either a muscular or a neuromuscular affair.

8. Artificial rhythm: We have not found it possible to produce rhythmical contractions of approximately uniform extent by repeated periods of vagus stimulation.

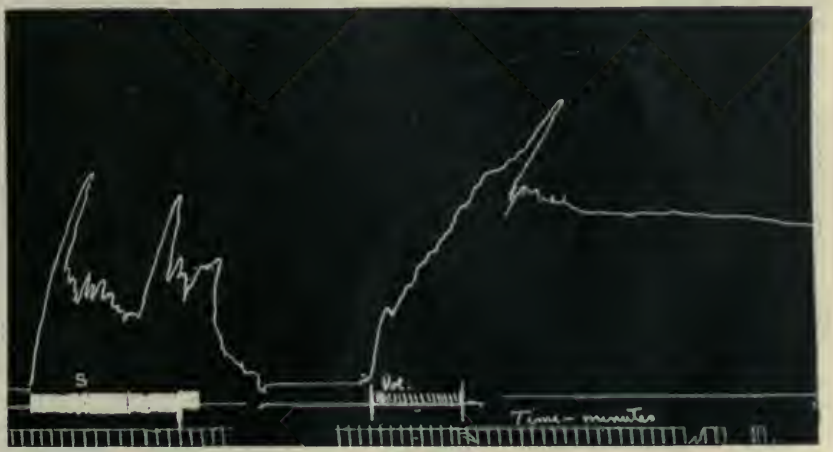

Fig. 4. Isolated strip of muscle of turtle stomach suspended in oxygenated Ringer's solution. *S*, Induced shocks applied directly to the strip; *Vol.*, passage of voltaic current through the isolated muscle strip.

These characteristic effects on the turtle stomach of vagus stimulation are not due to temporary asphyxia of the stomach due to simultaneous inhibition of the heart, for it requires hours of asphyxia to abolish vagus action, and these effects described can be induced for a time after ligation of the arteries from the heart. Furthermore, none of these are directly dependent on possible simultaneous inhibitory influences exerted through the splanchnic nerves for they can be elicited in the dog after cutting the splanchnic nerves and in the turtle after destruction of the spinal cord.

In contrast to the preceding, if the muscle strip be directly stimulated the following effects may be obtained:

1. Induced shocks at a rapid rate (6 to 40 per second) are followed by a contraction which persists as long as stimulation is continued (at least 15 minutes) (fig. 4).

2. By slow rate of stimulation we have not succeeded in inducing relaxation.

3. Continuous passage of a voltaic current leads to a tetanic contraction of the stomach which may continue during the flow of the current and also for an hour or more after the stimulating current is removed (fig. 4).

4. A single shock has given us no muscular contraction.

5. The muscle strip is much more resistant to asphyxia. Thus after the animal has been asphyxiated until stimulation of the vagus no longer induces contraction, on direct stimulation of the muscle itself, contractions can be obtained.

6. When the body temperature has been lowered until motor responses fail from vagus stimulation, direct stimulation of the muscle is followed by contraction.

7. An artificial rhythm is easily induced in the muscle strip by repeated short intervals of stimulation with the induced current.

DISCUSSION

From these experiments it is evident that any theory of the tonic action of the vagus on gastric motility must take into account the following considerations:

1. The vagus may have either an excitatory effect on gastric motility, or an inhibitory influence on gastric tone and contractions, in both dog and turtle, and according to Langley (2) and to Page May (3) the same is true of the rabbit, cat and monkey.

2. In the turtle no method of increasing gastric tonus is available by stimulation of the vagus.

3. Inability to induce a tetanic contraction in the turtle by vagus stimulation is dependent on the production of fatigue at the vagus synapses or refractory condition within the gastric nerve plexuses, and not to a peculiar condition of the muscle itself.

4. Lowering of temperature and asphyxia abolish the response of contraction to vagus stimulation, by acting on some part of the intrinsic plexuses rather than on the muscle tissue.

5. The inhibitory effects of vagus stimulation are not so susceptible to fatigue as the motor effects.

Page May has pointed out that no evidence of a tonic vagus effect is available in anesthetized mammals. Carlson (5) speaks of the inability to reflexly augment gastric tone or motility. As pointed out in this paper, relatively intense stimulation is required to overcome the resistance to conduction of the intrinsic plexuses. Drugs which are supposed to stimulate the medullary centers (picrotoxin and apomorphine) in the dog and rabbit have given only inhibition, and in the turtle temporary stimulation followed by depression (6). These considerations therefore raise the question as to whether or not the vagus normally exerts a tonic stimulating action on gastric motility. On the other hand, almost incontrovertible are the facts that stimulation of the vagus with the ordinary tetanizing induced shocks induces a short period of contractions, and the observations of Cannon (4) and Carlson (5) that sectioning both nerves leads to depression of gastric tonus. The only possible criticisms of the later results might be that there is no proof of nervous stimulation in the normal animal as intense as is required experimentally to cause contractions, and that depression of tonus after vagotomy may be secondary to some other disturbance in the viscera, perhaps sphincters, secretions, etc. The explanation of the paradox involved is to be sought perhaps in the further study of the properties of the intrinsic plexuses and conduction between the vagus and these plexuses.

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COMPARATIVE STUDY OF ETHANOL, CAFFEINE AND NICOTINE ON THE BEHAVIOR OF RATS IN A MAZE

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Received for publication February 27, 1921

The influence of drugs on psychological functions and on animal behavior has been very little studied by either psychologists or pharmacologists. The senior author in conjunction with several collaborators has found it convenient and productive of valuable results to study the pharmacological effects of drugs in this respect on albino rats in the circular maze. This apparatus enables the investigator to study not only the neuromuscular coördination of the animals, but also such specifically psychological phenomena as rate of learning, memory-habit and general behavior of the animals. Furthermore, in case of drugs, the maze offers, in the opinion of the authors, a convenient and valuable method of detecting the earliest and mildest symptoms of narcosis, using the latter term in its broadest sense, namely, as denoting a loss of normal responsiveness and automatic activity of the living organism. In this way Macht and Mora (1) have investigated the effects of opium alkaloids on albino rats; and again Macht and Bloom have made a similar study of the antipyretics, in one research (2), and have analyzed the cerebral effects of cocaine in another (3). In the present communication the authors propose to describe the results of a comparative study of three drugs which play an important rôle, not only in pharmacology, but also in social and personal hygiene—ethanol, caffeine and nicotine.

Description of the maze. A full description of the maze with illustrations has been published elsewhere (1), (2). Here only a brief recapitulation is made. The circular maze is made with wooden base and aluminum walls. The base is 150 cm. in diameter and 4 cm. in thickness. Its upper surface is marked off by grooves into a series of concentric circles. The diameter of each of the circles is as follows, beginning with the outermost one: 140 cm., 100 cm., 80 cm., 60 cm., 40 cm. and 20 cm. Into the circular grooves are inserted sheets of aluminum

18.5 cm. high and 0.8 mm. thick. Each strip of aluminum is cut just 10 cm. shorter than the length of the circular groove into which it is to be fitted, thus giving an opening into the alley. By means of this arrangement it is possible to slide the aluminum around in its groove and thus place the entrance in any desirable position. In the present investigation the 7 openings or entrances to the alleys were placed in such a position that the rat had to make alternate turns to right and left before reaching the center of the maze. In addition to the doors or openings, the alleys were provided also with obstructing partitions which formed a number of blind cul-de-sacs. A wire screen prevents the animals from crawling over the top. A camera lucida attachment to the maze has been invented by Professor Watson and is also described in full in the previous papers by the senior author. By means of this camera lucida attachment, the movements of an animal in the maze can be traced upon white paper with a soft pencil. Such tracings are especially useful in the study of the learning of the maze problem, such as has been done by Miss Hubbert and others. In the present investigation, where the effect of drugs on the behavior of the animals was studied after the rats had been trained, the use of this attachment was not essential and it was therefore dispensed with in a great many of the experiments.

The study of the behavior of the rats in the circular maze is begun by placing an animal in the center of the maze and feeding it for three successive days. During these three preliminary feedings, which last from 10 to 15 minutes, the entrance is blocked off so that the animal may not roam around. On the fourth day the rat is placed in the cage, then the trap-door is raised and the animal allowed to enter the first alley. The animal then gradually learns to find its way to the center of the maze, when it is taken out and the experiment is repeated. Generally three trials are made on each day. For work with the maze albino rats, which are tame, must be employed. The animals must be handled gently with the hands and under no circumstances must they be picked up with forceps or similar instruments. The most suitable animals are found to be rats approximately 60 to 90 days old. Older animals are apt to be sluggish, while very young rats do not learn the maze problem so readily. Ordinarily the albino rats learn the maze problem in about 2 weeks, and sometimes within a shorter period of time. An animal is considered to have solved the maze problem when it has learned to find its way into the center of the maze by the shortest route, that is, without any errors, on three successive trials. The technic of training is described in detail by Hubbert (4).

Analysis of the data furnished by the maze. In studying the effect of drugs, the maze problem can be utilized in two ways. Animals may be subjected to the influence of the drug action first and then trained in the maze with the purpose of ascertaining the effect on the rate of learning. Again, animals may be first taught to solve the maze problem and then the effect of a drug is studied in reference to its influence on their behavior, memory-habit, etc. Furthermore, other data can be obtained from the maze, after administering drugs to rats, which show the effect on neuromuscular coördination and various somatic changes. As to exactly what the mechanism of learning the maze problem may be, the explanations given by various experimental psychologists differ widely. Among the hypotheses which have been advanced to account for the reintegration of conduction paths in learning, there are at least three which stand out as rather opposed to one another in respect to the neural processes which they imply (5). The hypothesis suggested by Ladd and Woodworth (6) assumes inhibition of successive activities as the fundamental process which results in the selection and fixation of random activities. The second hypothesis, given by Angell and others (7), assumes nervous reinforcement as the fundamental process by which successive acts become linked together in habit-formation. The third hypothesis, that of Watson (8), depends chiefly upon the chance spreading of nervous excitation, or the simultaneous activation of two afferent pathways in such a way that the final common part of one is able to divert the discharge of the other and so bring about a permanent connection between itself and this afferent path. These hypotheses by no means exhaust the theoretical considerations of the maze problem (9). For the study of drug action, however, the various theoretical considerations are of secondary importance and the data obtained are of a much more definite nature, as will be seen from the following exposition.

Experimental data. In the present investigation a total of 40 rats was used. Most of the animals were males from 2 to 6 months old, a few females were also used. The drugs studied were dissolved in sodium chloride solution of 0.8 per cent and given by injection immediately after three normal trials were made in the maze. At the expiration of about 30 minutes the effects of the drugs were investigated and additional readings were made one or more hours later. Wherever possible the effects of the three drugs were studied on the same individuals, though, of course, not on the same days.

Effect of ethanol. Two to 4 per cent solutions of absolute ethanol in saline were employed. These were injected in some cases intraperitoneally and in other cases intramuscularly. No difference in the effects was noted between the two methods of administration. Control experiments were made with saline solution on the one hand and with distilled water on the other. The most striking feature in connection with the ethanol action noted, was its comparative low toxicity. One, 2, 3 and even more cubic centimeters of a 2 per cent solution

TABLE 1

Effect of ethanol

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
	gm.							
I	4.0	38	1	29	0	25	0	No effect
II	10.5	13	0	14	0	14	0	No effect
III	12.0	20	0	19	0	18	0	No effect
IV	12.0	17	0	26	0	14	0	Doubtful effect
V	12.0	23	0	16	0	16	0	No effect
VI	13.0	21	0	20	0	20	0	No effect
VII	16.0	15	0	16	0	15	0	No effect
VIII	25.0	31	0	20	0	28	0	No effect
IX	32.0	28	1	26	1	24	1	No effect
X	36.0	16	1	15	0	15	0	No effect
XI	52.0	14	0	20	0	15	0	Doubtful effect
XII	65.0	16	0	23	1	34	0	Depression
XIII	65.0	20	0	23	0	34	1	Depression
XIV	80.0	17	0	19	0	19	0	Slight depression
XV	80.0	23	0	25	1			Depression
XVI	90.0	22	0	22	0	27	0	Depression
XVII	95.0	21	0	39	1			Depression
XVIII	95.0	14	0	102	2	33	1	Marked depression
XIX	105.0	16	0	85	5	35	2	Marked depression
XX	125.0	22	1	63	4			Marked depression

were found to produce no appreciable effect on the rats as far as could be ascertained from their behavior in the maze. The smallest doses of ethanol which were found to produce a change were from 60 to 65 mgm. by weight of the drug. This effect was indicated by a slowness of progression, slight neuromuscular incoördination and disturbances in memory-habit as shown by errors committed. Table 1 expresses some of the data obtained with this drug. No primary improved or stimulation effect in running time or discrimination was noted after injections of ethanol in any of the experiments.

Effect of nicotine. Nicotine tartrate was dissolved in normal saline solution. This was injected either intraperitoneally or intramuscularly. The drug was found to be much more depressant than either ethanol or caffeine. The smallest dose to produce any effect was found to be 0.02 mgm. Such a dose of the tartrate is equivalent to about 0.0066

TABLE 2

Effect of nicotine tartrate

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
I	0.010	38	0	35	0	35	0	No effect
II	0.015	26	0	27	0			No effect
III	0.015	27	0	18	0	20	0	No effect
IV	0.016	19	0	22	0			Doubtful effect
V	0.02	18	0	49	0	56	0	Depression for 2 days
VI	0.02	15	0	35	2	20	3	Depression
VII	0.02	29	0	38	1			Depression
VIII	0.02	27	0	29	1			Depression
IX	0.025	18	0	26	0			Depression
X	0.025	15	0	12	0	21	0	Depression on next day
XI	0.03	17	0	19	0	Stalled		Depression
XII	0.03	44	0	80	3			Depression
XIII	0.03	21	0	30	1			Depression
XIV	0.05	13	0	72	2			Depression
XV	0.05	22	0	15	0	16	0	Depression on next day
XVI	0.06	17	0	16	0	16	0	Depression on next day
XVII	0.06	30	0	40	1	70	4	Depression for 2 days
XVIII	0.06	20	0	124	3			Marked depression
XIX	0.075	62	0	Stalled		Stalled		Marked depression for 2 days
XX	0.100	22	0	43	2	31	0	Depression

mgm. of the alkaloid nicotine itself. The injection was found to produce depression in the behavior of the animals as indicated by slower progression and failure in discrimination and memory. In no case was there any primary stimulation noted. Another striking feature of the action of nicotine was seen in the presistence or duration of the effects. Distinct impairment in the psychological functions of the rats was generally noted on the following day and even later. Table 2 indicates some of the results obtained with nicotine.

TABLE 3

Effect of caffeine

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
I	0.06	20	0	21	0	21	0	No effect
II	0.10	22	0	21	0	24	0	No effect
III	0.12	13	0	16	0	15	0	No effect
IV	0.12	18	0	41	0	23	0	Slight depression
V	0.30	15	0	15	0	15	0	No effect
VI	1.00	18	0	17	0	20	0	Slight depression
VII	1.00	18	0	17	0	17	0	No effect
VIII	1.25	15	0	14	0	15	0	No effect
IX	1.60	15	0	14	0	16	0	No effect
X	2.50	19	0	19	0			No effect
XI	3.00	18	1	18	1	18	0	Jerky movements
XII	4.00	15	0	16	0	16	0	Jerky movements
XIII	5.00	15	0	30	1	16	0	Depression
XIV	5.00	15	0	16	0	16	0	Doubtful effect
XV	5.00	51	1	47	1	29	0	No effect
XVI	6.00	20	0	30	1			Depression
XVII	6.50	18	0	20	0	20	0	Depression
XVIII	7.00	24	0	24	0	17	0	No effect
XIX	7.50	33	0	36	2			Depression
XX	7.50	15	0	70	4			Great depression Straub P.
XXI	8.00	18	0	28	1			Depression
XXII	8.00	25	0	19	0			No effect
XXIII	8.50	15	0	15	0			No effect
XXIV	9.00	17	0	22	0			Marked depression
XXV	10.00	20	0	20	0			No effect
XXVI	10.00	17	0	27	1			Marked depression
XXVII	13.00	15	0	Stalled		Stalled		Great depression for 2 days. Straub P.
XXVIII	14.00	27	0	Stalled		Stalled		Great depression. Diuresis
XXIX	15.00	16	0	Stalled		Stalled		Straub P. Death after 2 days
XXX	15.00	15	0	Stalled		Stalled		Straub P. Slow recovery after 5 days
XXXI	25.00	17	0	Dies				
XXXII	30.00	16		Dies				

Effect of caffeine. The drug employed was the alkaloid caffeina U. S. P. This was dissolved in saline to make solutions of 0.2 per cent and 0.5 per cent and the drug was in this case also injected either intraperitoneally or subcutaneously. In the present investigation the authors noted no primary improvement or any stimulation effect after injection of the drug as indicated by a shortening of the running time or by a lower number of errors. Occasionally there was noted, however, a nervousness or excitability of the animals as indicated by a jerkiness or suddenness in their movements. When studied in the maze however the effect on the animals, even in such cases, could not be called a stimulating one, but rather of a depressant character. The smallest doses of the drug to produce any change were 5 to 6 mgm. per 100 grams of weight. Such doses were found to retard the progression of the animals and produce a depression in their memory-habit, as may be seen from table 3.

DISCUSSION

A comparative study of all the data obtained by the authors with the three drugs examined reveals the fact that as far as the effects on the intelligent behavior of rats in the maze are concerned they are all depressant, that is, when given in sufficient quantity they impair the running time of the animals and the accuracy of discrimination or choosing the proper path or so-called memory-habit. The authors were not able to discern in any case, even after very small doses of the drugs examined, any stimulating effect in this respect. The only drug of the three in question, as far as they know, that has been experimented with in the maze, was caffeine. Lashley (5) investigated the effects of small doses of caffeine when injected into rats on their behavior in the maze: and his results show also that this drug does not produce a real stimulation.

It is interesting to note that of the three substances studied by the present authors, ethanol is much the least toxic. Large doses of this drug must be injected in order to produce a depressant effect. The smallest dose giving a definite depression was found to be 80 mgm. per 100 grams weight of the animals; although in some individuals doses of 60 mgm. also suggested a slight depressant action. Nicotine was by far the most toxic of the three drugs. It was interesting to note however that the effects of this poison did not manifest themselves always immediately after injection. The depression came on sometimes much later and in almost all cases persisted for at least 2 days.

The effects of caffeine were almost as unexpected as those of ethanol. Whenever this drug was found to produce an effect, such effect was of a depressant character. The smallest doses found to be effective in this way were 4 or 5 mgm. per 100 grams weight of the rats. Such doses while never producing an improvement in the absolute running time of the animals, or in their accuracy, often produced a spasmodic jerkiness in the muscular movements of the animals, so that the animals would start off to run at a very rapid gait. Such movements, however, were generally combined with an increased number of errors, so that the total time consumed in reaching the center of the maze was prolonged. After large doses of caffeine the impairment in the behavior of the rats was much more marked, but in the case of this drug, unlike the experiences with the other two drugs, there was a greater number of individual variations. It would seem that young rats were more sensitive to the action of the drug than older ones, but this observation requires further corroboration. After large doses of caffeine such as 10 to 20 mgm. per 100 grams weight, the impairment in the neuromuscular functions and the cerebral response of the rats lasted for many days, sometimes for 2 weeks. It was further noted that after large doses of caffeine the rats exhibited a stiffening of the tail, that is, the so-called Straub phenomenon. This was not surprising inasmuch as the analysis of the mechanism of this phenomenon shows that it is produced by a muscular spasm of the sphincters of the bladder and the rectum (10).

A comparison of the relative toxicity of ethanol, caffeine and nicotine obtained in the present investigation with the rats naturally invites a comparison of the relative toxicity of these drugs in man. Thus it would be interesting to determine the relative quantities of the three pharmacological agents, ethanol, caffeine and nicotine, which are absorbed by man on imbibing various beverages or on smoking cigars and tobacco in other forms, on the one hand, and comparing the relative toxicity of the same drugs for man, on the other hand. Such a discussion is however beyond the scope and purpose of the present paper.

SUMMARY

1. The effects of ethanol, caffeine and nicotine were studied on the behavior of rats in the circular maze.

2. It was found that none of the drugs studied by this method produced an improvement or stimulation in the behavior of the rats.

3. It was found that all of the three drugs when injected in sufficient quantities produced a depressant effect, nicotine tartrate being the most toxic, caffeine coming next and ethanol being the weakest in this respect.

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THE MODIFICATION OF THE CROSSED EXTENSION REFLEX BY LIGHT ETHERIZATION AND ITS BEARING ON THE DUAL NATURE OF SPINAL REFLEX INNERVATION

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Received for publication February 28, 1921

In the course of a class demonstration on reciprocal innervation in the decerebrate cat an interesting effect of light etherization on reflex behavior was recently noted; it appeared to be of such significance in its bearing on the physiology of the spinal nerve centers as to warrant further study.

The normal dominant reflex responses to stimulation of an afferent nerve in a hind limb are the flexion reflex and the crossed extension reflex; the flexion reflex consists in reflex excitation of the flexor muscles and inhibition of the extensor muscles in the same limb as the stimulated nerve; the crossed extension reflex consists in reflex excitation of the extensors and inhibition of the flexors in the opposite hind limb (1, p. 108). Many researches have pointed to the view that in general the stimulation of an afferent nerve tends to provoke two antagonistic central effects. Thus when an afferent nerve in a hind limb is stimulated the effect is to produce not simply the dominant reflex responses just described, but also a tendency in an exactly opposite sense which may or may not be latent (2); that is, a tendency to produce excitation of extensors and inhibition of flexors on the same side as the stimulated nerve. Sherrington and Sowton (3) using a decerebrate cat, showed that whereas the usual and universally obtainable result of stimulating an afferent nerve in the hind limb was to produce reflex inhibition of the knee extensor, vasto-crureus, they could under certain conditions change this result into the direct opposite, namely, reflex excitation of the extensor muscles, by changing the strength and form of the electrical stimulus, provided the extensor muscles exhibited at the outset a fair degree of reflex tonus. Strong faradization regularly produced inhibition; weak faradization sometimes produced excitation, and weak alternating currents with a frequency of 20 cycles a second and con-

sequently much less abrupt onset than induction shocks, were especially efficacious in producing the excitatory response. In short, strong and abrupt stimuli regularly provoked inhibition; weak stimuli, especially consisting of currents with gradual onset, provoked reflex excitation, if a sufficient background of reflex tonus existed.

Sherrington has investigated the phenomenon of extensor rebound following inhibition (4). This is present in a large proportion of decerebrate preparations, and consists of a fairly vigorous reflex contraction of extensor muscles following the cessation of stimulation applied to an afferent nerve in the same limb, when their tonic contraction has been inhibited during the application of the stimulus. This response was first likened to the setting free of a pent up stream of energy (1, pp. 203–212), but after Sherrington and Sowton had found that an immediate excitatory effect was being produced by afferent stimulation, replacing under certain conditions the usual inhibitory effect, they proposed an explanation of this rebound contraction as being due to a twofold reflex influence exerted by the stimulus during its application, and the greater persistence of the excitatory effect after its withdrawal (5). Sherrington's observations on the rebound contraction include additional reasons for this view, to which attention has already been called (6, p. 160). It may well be emphasized that the flexion reflex is notably characterized by its brief latency and brief after-discharge, in short, by its approximate limitation to the duration of the applied stimulus. The crossed extension reflex on the other hand is strikingly characterized by a longer latency and a very much longer after-discharge (1, p. 77). One of its most salient features is the fact that it outlasts the exciting stimulus usually by many seconds. It is also well established that the flexion reflex is usually much the more easily provoked of the two, and if with equally strong stimuli an attempt is made to provoke the two simultaneously the flexion reflex normally completely dominates in the reflex effect. Therefore if in an afferent stimulus a tendency to produce a central effect similar to the crossed extension reflex were to exist together with a tendency to produce the flexion reflex, we should expect exactly what we find in the usual response to stimulation of a single afferent nerve in the limb under observation; namely, flexion during application of the stimulus and, following its cessation, the appearance of the extensor effect which is well known to be characterized by its after-discharge or tendency to outlast the stimulus.

Graham Brown (7) has reported a rhythmic reflex response to simultaneous application of two antagonistic stimuli; that is, to stimulation

of afferent nerves in both hind legs. At the same time I reported a similar but more rapid rhythm in response to the same procedure (8). In certain preparations I found a similar rhythmic response to the stimulation of a single afferent nerve, and was led to regard this as evidence of the two antagonistic elements in the central effect. Sherrington (9) found that in the cat or dog a small dose of strychnine converted the usual inhibitory effect on the knee extensor of stimulating an afferent nerve, into an excitatory effect. He found that this change could then be undone by administration of chloroform or ether. Subsequently Sherrington and Sowton (10) reported that in the decerebrate cat the usual excitatory effect on the extensors of stimulating a nerve in the opposite leg was changed by chloroform into an inhibitory effect. This reversal appears before the effect of chloroform has gone far enough to abolish the reflex tonus in the extensor muscles which is necessary as a background for inhibition. Both the strychnine and chloroform reversals are rendered more intelligible by the concept of a twofold effect always present in response to any afferent stimulus. The conversion of a reflex effect into its exact opposite by a drug is a puzzling phenomenon until we recognize that two antagonistic elements in the central disturbance are always present; then the conversion of inhibition into excitation by strychnine appears as the abolition of the inhibitory content, enabling the excitatory content to come into evidence; and the conversion of excitation into inhibition by chloroform may similarly be interpreted as due to the abolition of the excitatory content of the disturbance.

OBSERVATIONS

The initial observation which struck me as being significant in connection with the above considerations and which led me to seek a repetition of it in other experiments, was as follows:

A cat was decerebrated under deep ether anesthesia with the decerebrating device described by Miller and Sherrington (11), the transection being about 3 mm. in front of the anterior edge of the anterior colliculi. Instead of a steady state of decerebrate rigidity the animal showed considerable spontaneous activity in the fore limbs, neck and trunk. In my experience, this is usually the result of a transection farther forward than that which gives typical decerebrate rigidity. Therefore, the animal was again etherized and a fresh transection made at the front edge of the anterior colliculi. The animal still showed much the same sort of activity as after the first transection, but was nevertheless pre-

pared for the demonstration of reciprocal innervation of antagonistic muscles. Stimulating electrodes were applied to the two sciatic nerves and all muscles of the right hind leg were paralyzed by nerve section except the vasto-crureus and the semitendinosus. The former was left with its normal attachments so that its contractions and relaxations should be shown by extension and flexion of the knee joint; the tendon of the semitendinosus was cut from its insertion and connected with a muscle lever to show separately the simultaneous behavior of a knee flexor. The reflexes were demonstrated, but the spread of reflex response to muscle groups in the anterior part of the animal was marked, and the almost constant activity of the fore limbs was distracting. Therefore the animal was again lightly etherized and a third transection was made through the middle of the anterior colliculi and close to the front edge of the posterior colliculi. After this transection the animal developed good typical decerebrate rigidity in the fore limbs, but little or none in the hind limbs. The sprawling activities disappeared, and the reflexes became more regular.

Shortly after this third transection, and while the animal was still under the effects of ether, it was noted that the crossed stimulus,—that is, stimulation of the left sciatic nerve,—even if strong, caused, instead of the usual full extension, only partial extension during stimulation, but a marked increase of extension when the stimulus ceased. This effect was observed repeatedly. As the ether wore off it became less pronounced. The effect appeared also to fatigue. If a second extensor stimulus was applied while the after-discharge of the first was still pronounced, the effect was not present. After a few minutes the animal was etherized again, and the effect became distinctly more pronounced, again becoming less so as the animal came out of ether. A third etherization produced the same result. No rebound contraction followed the inhibition of the extensors on stimulating the right sciatic nerve (flexion reflex), unless this reflex was evoked shortly after the cessation of the crossed stimulation; that is, during the time normally occupied by the after-discharge of the crossed extension reflex.

It was not feasible to prepare the necessary apparatus for making a permanent record from this animal, but five cats were subsequently decerebrated and prepared for recording this effect. In every case the transection was made in the neighborhood of the colliculi, usually about the middle of the anterior colliculi. In one case the transection was very close to the anterior edge of the cerebellum. In the first three experiments, including the one just described, the stimulating apparatus

consisted of a small inductorium such as is used for class work, operated by a buzzer interrupting the primary current approximately 50 times a second. The coil was not calibrated and no attempt was made to determine the strength of primary current. In the last three experiments a Berne coil was used which had been calibrated for break shocks in accordance with Martin's scale (12, p. 55). The primary current was measured with an ammeter and was interrupted by means of a rotary device so designed that the frequency of interruption and the duration of closure at each contact could be independently regulated at will.

The construction of this interrupter is as follows: On the shaft of a twelve volt D. C. motor is mounted a cylinder with six copper segments with which contact is made by two light copper brushes so designed as to have a natural period of vibration much more rapid than any frequency with which the apparatus is to be used. The six segments provide for six interruptions per revolution, and one of the brushes is so arranged that its position with respect to the other can be varied in such a way that the duration of closure each time the circuit is closed may be varied from 0 to 92 per cent of the time from the beginning of one closure to the beginning of the next. Coupled with this motor is a magneto connected with a voltmeter to serve as a speed indicator, and in the armature circuit is a carbon rheostat by which the speed may be regulated with considerable accuracy. It is possible to obtain frequencies of interruption from 25 to 270 per second. Tests with the string galvanometer when the apparatus was new showed perfectly maintained closure at all speeds tested. Recently a similar test has shown that at fairly high speeds one closure in each revolution, that is, one in six, is not perfectly maintained, and may thus result in the production of extra induction shocks. In these experiments the movable brush was so placed as to give 70 per cent of closure; that is, the break shock would occur after 70 per cent of the time had elapsed from one make shock to the next. This adjustment has been adopted as standard because Erlanger and Garrey (13) showed that the relatively great duration of make shocks may result in a reduction of the intensity of break shocks if the latter follow too close after them.

In the first four experiments the operative procedures were carried out exactly as described in the case of the first experiment. In the last two experiments no stimulating electrodes were applied to the right sciatic nerve, but it was cut at the hip, including the nerve to the hamstring muscles. All branches of the anterior crural nerve were cut

except those to the vasto-crureus muscle. The psoas muscles were cut, and as many branches of the obturator nerve as could be conveniently reached. In every experiment the femur was clamped vertically so that the foot would fall by gravity if the muscle relaxed. Thus all motions which could be recorded on the drum were excluded except those due to contraction or relaxation of the vasto-crureus muscle.

A strength of stimulus was selected at which a strong crossed extension reflex was obtained. A record of this was then obtained on a slowly moving drum, the time of application of the stimulus being recorded by a signal magnet. The drum was stopped and synchronous ordinates were obtained by again stimulatiug the nerve while the drum was stationary. Thus by measuring back from one of these synchronous ordinates it was possible to determine the point in the myogram corresponding with the cessation of the stimulus. After recording a few normal responses the animal was etherized and at intervals the response to similar procedure was obtained. The duration of stimulation was varied somewhat. In general it was maintained until the contraction of the muscle had practically ceased to increase. When the cessation of the stimulus was followed by the marked increase noted in the first animal, this would appear conspicuously on the record. As soon as the effect had appeared, or failing that, as soon as the crossed extension reflex had become reduced by the effect of ether to a very weak contraction, the ether was withdrawn and a series of records was taken as the effect of it was wearing off, until the reflex had returned to its normal character as unaffected by the anesthetic.

In every animal investigated the effect was found. In three out of the six it was marked; in the remaining three it was usually observable, but was not nearly so striking as in the other three. Figures 1 and 2 show typical records obtained from two of those animals in which the effect was marked. Figure 1 is taken from the animal in which the transection was made close to the cerebellum, and in this animal the effect was more marked than in any other. Ether had been given for 3 minutes from an ether bottle connected with a tracheal cannula. Four minutes after its withdrawal the first stimulus shown in the record was applied, the strength of stimulus being much greater than that required to produce a vigorous reflex before etherization. Just before this observation a somewhat weaker stimulus had failed to produce any reflex response. The second stimulus shown in the record was applied one minute after the first, the next two with intervals of 1½ minutes preceding them, and the last two with intervals of 2 minutes preceding them.

Fig. 1. Crossed extension reflex, vasto-crureus muscle; 4½ hours after decerebration (see text). In this and all subsequent figures including figure 6, ascent of the myograph line means contraction of the muscle. All read from left to right. Stimuli from uncalibrated coil. Time in seconds at the top.

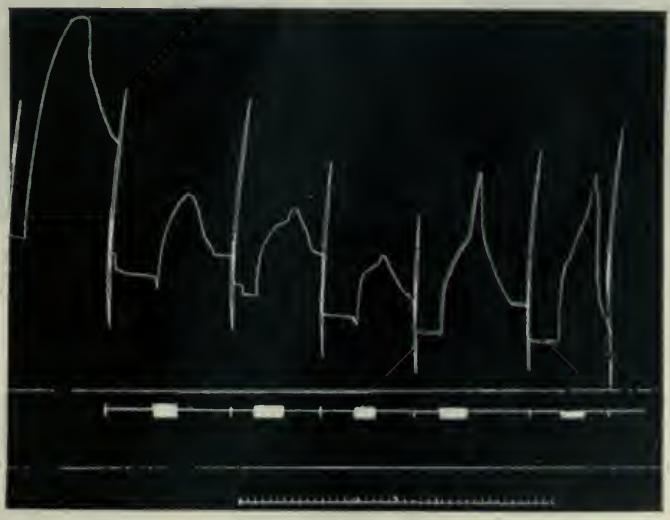

Fig. 2. Arrangement the same as figure 1; 2 hours after decerebration. Stimuli in first two observations, 88 Z units; in the remainder, 138. Primary current interrupted with rotary circuit-breaker 75 times a second. The speed of the drum was constant throughout. Time in seconds shown at bottom in part of the record.

Thus the last record, which shows an approximately normal crossed extension reflex with only a slight trace of the effect under discussion at the end, was obtained 12 minutes after the withdrawal of ether.

Figure 2 shows the effect in another of the preparations in which it was marked. The first contraction shown is the normal reflex, before ether was administered. Immediately after this observation ether was applied for 40 seconds. The second stimulus was applied a minute after the first. Between the second and third observations the strength of stimulus was increased, and the greater strength continued throughout the remainder. The last observation in the series was made 4 minutes after the first. Here a recovery from the ether has already begun to make the effect less pronounced.

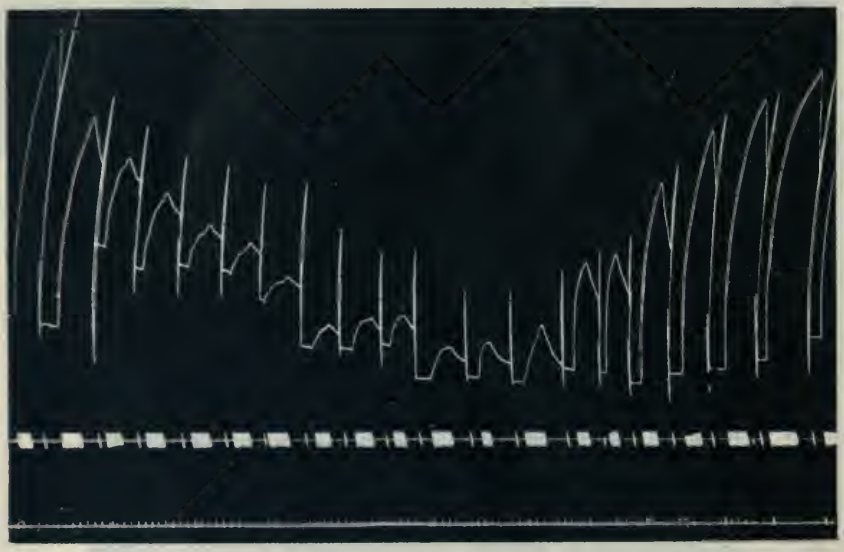

Fig. 3. Two and one-half hours after decerebration. Stimuli 170 Z units throughout. Interruption of primary current as in figure 2. Time in seconds at the bottom.

Figure 3 shows a typical series of observations from one of the animals in which the effect was least marked. This animal remained in good condition for a long time, and observations were made throughout the course of ten successive etherizations. The effect appeared to a slight extent, as is shown in the figure, during the onset of seven out of these ten etherizations. It failed to appear during the recovery from

ether. Ether was administered between the first and second observations shown in the figure, and withdrawn between the sixth and seventh observations. Fifteen minutes elapsed from the first to the last observation in the series and the intervals between the separate observations varied from 15 seconds to 2½ minutes. The strength of stimulus was maintained constant throughout the series.

DISCUSSION

General considerations. Graham Brown (14, fig 5, p. 348) has recorded a reflex response which shows superficially a close resemblance to the effect here described. This was obtained in a low spinal preparation 7½ hours after cutting the spinal cord. Contralateral stimulation produced extensor contraction, followed, on withdrawal of the stimulus, by a marked increase in the amplitude of contraction. In his observation there was no narcosis and the effect appears to have depended on a low spinal transection, a procedure which is well known to swing the "neural balance" away from extension toward flexion. He has also described a "narcosis progression" (15), (16) or rhythmic alternation between flexion and extension, occurring reciprocally in the two hind limbs under narcosis and independent of afferent stimulation. This may be related in some way to the effect here described, but the relation does not appear to be very close since the effect here described shows no evidence of rhythm, but merely an increase in extension whenever the stimulus ceases.

The partial abeyance, under the influence of ether, of the crossed extension reflex during the application of the stimulus which normally provokes it, appeared to me significant as illustrating an effect similar to the chloroform reversal of Sherrington and Sowton (10). Reflex excitation appears to have been in part converted into reflex inhibition. The release of reflex excitation on cessation of the external stimulus at once suggests the rebound contraction of extensors following their reflex inhibition in the majority of normal decerebrate preparations. The picture is most easily interpreted on the assumption that the external stimulus produces two antagonistic central influences—extensor and flexor; that is, with respect to the extensor motor neurones—excitatory and inhibitory (8). On this view the effect of ether is to narcotize the extensor element more than the flexor, and thereby prevent the usual dominance of the extensor effect during the application of the stimulus. But since extensor reflexes are notably characterized by a prolonged

after-discharge as compared with flexor reflexes (1, p. 30), the increased extensor activity following the withdrawal of the stimulus may be simply the expression of this difference in after-discharge. This view is in harmony with the interpretation of the reflex rebound proposed by Sherrington and Sowton already referred to in the introduction. Graham Brown has shown that the crossed extension reflex is abolished by general narcosis more readily than the flexion reflex (16, p. 30). This fact lends support to the view outlined above, for in the dual effect the extensor element normally prevails, and yet under ether it is visibly restrained by the flexor element as long as the stimulus lasts, and only becomes wholly dominant when the stimulus ceases.

The effect here described does not appear to have occurred in the experiments of Sherrington and Sowton in which they showed a conversion of reflex excitation into reflex inhibition by the action of chloroform (10). It is possible that this is due to a difference between the two drugs, but it seems to me more likely that the difference depends on the degree of narcosis or on the intensity or rhythm of stimulation. That the absence of this effect in their experiment may be due to the weakness of their stimulus as compared with mine, is rendered probable by the fact that their stimulus is expressly stated to have been weak, whereas in these experiments it was found necessary to use stimuli stronger than were required to produce a powerful crossed extension reflex before narcosis.

In their earlier paper they observed that merely changing the intensity of stimulation sufficed to cause a reversal of the central effect. Thus in their experiment with chloroform the stimuli may have been inadequate to excite the extensor mechanism in the central complex under the degree of narcosis then present, at least to the extent necessary to induce the requisite after-discharge. Sherrington (1, fig. 27, p. 77) has shown that the crossed extension reflex changes as the stimuli increase in strength chiefly by showing a much longer after-discharge; an observation which may be significant in this connection, since it is the after-discharge on which the effect depends.

Basis of analysis. The observations on which most of our knowledge of reflex behavior rests were made when the character of the nerve impulse, as revealed by the work of Lucas and Adrian (17), (18), (19), (20), (21), was unknown. It was customary to think of nerve impulses as streams of energy which could be graded in intensity by grading the strength of stimulus, much as one increases a stream of water by opening a faucet.

It is now well established that the nerve impulse obeys the "all-or-none" law (21); that the only gradations which can occur in a single response, aside from varying the number of conducting units excited, are due to the relative refractory phase (stage of recovery from previous response), unless there is a deranged state of the tissue through narcosis or other injurious condition. In any lapse of time beyond that required for a single response there is also the possibility of gradation in the frequency with which the responses follow each other in each conducting unit. There can hardly be a reasonable doubt that this law applies to all nerve fibers, afferent and efferent alike, since no morphological or functional differences have been found which indicate any divergence so profound as would be involved by an exception to this law.

It will be interesting to see how some of the phenomena of central antagonism, "neural balance," etc., can be interpreted in terms of this new conception of the nerve impulses of which the reflexes are built. In particular it will be of interest to see how far the explanation of reflex inhibition proposed by Lucas (17), (22) can be made to fit the facts of reciprocal innervation and the relationships observed between opposing central effects.

Gradation in reflex response strikingly follows gradation in intensity of afferent stimulus over a very wide range of intensities (23). Graham Brown (24), studying gradations in the reflex response of the tenuissimus muscle, concluded that each motor neurone must discharge with graded intensity. His stimuli were not single induction shocks, but a series of tetanizing stimuli; furthermore it has been shown (23, p. 205) that even a single induction shock of great strength may produce compound instead of simple stimulation. Thus the possibility of gradation in frequency is introduced and with it the possibility of central summation. His results therefore do not prove the existence of any other mode of gradation than those specified above. It has been shown in a previous paper (23, p. 211) that even after a single induction shock has been made strong enough to excite all the fibers of an afferent nerve, as judged by the fact that it provokes a maximal action current, further increments in the intensity of the induction shock can produce very great additional increments in the extent of reflex responses. The explanation of this seems to be compound stimulation. By this I mean that the stimulus provokes so profound a local disturbance that the local excitatory process persists through the refractory phase and is able to initiate a second and possibly a third propagated disturbance in a single nerve fiber. This must be borne in mind in appraising many

of the researches in which reflexes were studied under the effect of strong faradic stimulation.

We may start our analysis of reflex behavior with the assumption that all differences in afferent stimulation must depend ultimately on differences in the number of fibers stimulated and differences in frequency of impulses in the individual fibers, bearing in mind that in the case of strong stimuli the nerve impulses may have a higher frequency than the induction shocks which induce them, owing to the possibility of compound stimulation arising from individual shocks, and that at high frequencies the impulses will be of more or less subnormal (22) (and under certain circumstances (25), (26) supernormal) magnitude, according to the frequency.

Lutz (27), working on frogs, found that the average threshold of stimulation for the nerve-muscle preparation was about 4 Z units (Martin's scale) whereas the average threshold for the flexion reflex with electrodes similarly applied to an afferent nerve was about 8 Z units. By cooling the frog he raised the threshold for the nerve-muscle preparation by about 0.1 Z unit per degree C., whereas the threshold for reflex stimulation was raised nine times as much as this per degree C. (28). Since he worked with single induction shocks of threshold strength, and consequently almost certainly far too weak to produce compound stimulation in the nerve, each afferent fiber carried only a single impulse to the center; there was therefore no opportunity for summation of successive propagated disturbances. If individual reflex arcs were isolated so that each afferent fiber was connected with a single motor neurone and only one, then if such an arc were capable of transmitting a single impulse it should make no difference in the motor response of that arc how strong a stimulus was used, provided it was above threshold. The reflex preparation would then be analogous to the nerve-muscle preparation, but with one more junctional point in each conducting path. Under these conditions the difference in threshold which Lutz found between the nerve-muscle and the reflex preparations would depend on a difference in irritability between afferent and motor fibers. A difference as large as he found would on this assumption be surprising, but not impossible. But the great rise in the threshold of the reflex preparation on cooling as contrasted with that of the nerve-muscle preparation, would imply on any such basis a most improbable functional difference between afferent and motor fibers; it seems altogether unlikely that afferent fibers, morphologically so similar to motor fibers, should have nine times as large a temperature coefficient of threshold.

Accepting the all-or-none law and assuming isolated reflex paths, the only other possible explanation would be that cooling in every case established a central block in all the synapses except those which happened to be connected with those fibers in the afferent nerve which were relatively inexcitable or relatively inaccessible to the stimulating current. This involves a series of coincidences obviously most improbable. The most reasonable explanation seems to be that individual reflex arcs are not isolated, but that two or more afferent neurones converge at a single central neurone, internuncial or motor (cf. 28, p. 525, fig. 6). This does not necessarily imply that in a given collective reflex arc there are more afferent than motor fibers. It is conceivable that a group of ten afferent fibers might each make connection with every one of ten motor neurones. The extensive branching known histologically to exist in the grey matter would account for such interconnection to a high degree of complexity, and the large excess of afferent over motor neurones favors it. I have elsewhere shown (29) physiological evidence that individual motor neurones are accessible to afferent impulses from fibers in different nerve trunks which evoke the same reflex response. Impulses from two or more afferent fibers converging at synapses of a single motor neurone might suffice to set up in it an impulse or series of impulses which one approaching through a single afferent neurone would fail to do.

In making our analysis of reflex activity we may then take for granted the possibility of gradation in the frequency of impulses in each fiber and in the number of fibers involved, and furthermore the possibility of convergence of these fibers on individual central neurones.

Possible mechanism of reflex inhibition. The explanation of reflex inhibition proposed by Lucas (22), (17) is based on his analysis of the so-called Wedensky inhibition. Briefly stated, it is that in some portion of the conducting path involved in the reflex process, presumably the last internuncial neurone traversed by the disturbance before the motor neurone, the impulses follow each other with such frequency that each occurs during the relative refractory period following its predecessor, and consequently is subnormal; that the synapse through which the impulses must pass in order to reach the motor neurone constitutes a region of decrement through which these subnormal impulses cannot pass, although full-sized impulses such as would occur at a slower frequency of discharge could pass through. A neurone so occupied would establish an absolute block provided that only through it could the motor neurone be excited, for at all times it would be either absolutely

or relatively refractory and incapable of a larger response than those subnormal impulses already being conducted and extinguished at the synapse. In the subsequent discussion we may conveniently designate this the "pre-motor" neurone. *In such a neurone there would be correlated with the degree of decrement at the terminal synapse a critical frequency of nerve impulses above which the effect would be inhibitory, and below which, excitatory.*

The balancing of antagonistic central effects. In discussing this explanation Adrian (30, p. 45, cf. 17, p. 98) has mentioned as an objection Sherrington's observation (31) that if an excitatory and an inhibitory nerve are excited at the same time the effect produced is a simple algebraic summation of the two single effects (i.e., an intermediate degree of extension), and depends entirely on the relative strength of the two stimuli. It appears difficult to reconcile this fact with the proposed explanation, for (30, p. 45) "the frequency of the impulses in the central paths should be, if anything, greater when both nerves are stimulated than when the inhibitory nerve is acting by itself, and therefore the impulses should be still further reduced in intensity."

I have studied this same balancing of excitatory and inhibitory central effects in the vasto-crureus muscle of the decerebrate cat, using induction shocks from coils calibrated according to Martin's scale. In my experience stimulation of the afferent nerve on the same side as the extensor muscle observed, unless very weak, has always sufficed to produce complete inhibition, unless the central effect was complicated by a synaptic fatigue resulting from continued stimulation of that nerve (29, p. 112). Under similar experimental conditions Gregg and I found that induction shocks of 40 to 50 Z units were required to excite all fibers in the sciatic nerve of the cat as judged by the size of the resulting action current. It is desirable to know at what strength of inhibitory stimulus its effect can still be partly overcome by an excitatory stimulus.

I have examined a series of old records, some of which have been published (6), with a view to estimating the degree of inhibition, by measuring on the myograph the percentage of the total possible relaxation obtained by inhibitory stimuli of various strengths applied to the peroneal nerve. The relaxations have been measured in each case from the degree of contraction existing at the time the inhibitory stimulus was applied. In every experiment in the series the make shocks were subminimal, and the break shocks were delivered to each nerve at a rate between 40 and 50 per second. The results of this examination

are shown in table 1. For the sake of indicating when two or more observations were made on the same preparation, these are identified by the same numbers that were assigned to them in an earlier paper (6). These identification numbers are given in the first column. In the third column is mentioned the degree of decerebrate tonus existing at the time of the test, and to which was added the crossed extension reflex in all but three observations. In every case the knee was in approximately extreme extension when the inhibitory stimulus was applied. Preparation 5 was a high spinal preparation and therefore without

TABLE 1

PREPARATION	INHIBITORY STIMULUS, Z UNITS	TONUS	EXCITATORY STIMULUS, Z UNITS	PER CENT INHIBITION
3	17.0	Strong	No stimulus	65
3	18.0	Strong	No stimulus	89
3	17.0	Strong	45.0	22
3	18.0	Strong	45.0	30
3	24.0	Strong	72.0	95
3	26.0	Strong	72.0	35
3	26.0	Strong	72.0	78
3	26.0	Strong	72.0	45
3	26.0	Strong	72.0	30
3	26.0	Fair	72.0	78
3	26.0	Moderate	72.0	83
2	18.0	None	50.0	81
2	15.0	None	50.0	75
5	14.0	None	44.0	92
7	7.0	Slight	14.0	92
9	4.0	Medium	28.0	61
9	4.8	Good	No stimulus	82
10	5.8	None	6.4	95

tonus; all the others were decerebrate. The twelfth and eighteenth observations in the table are those appearing in figures 9 and 10 respectively in the previous paper (6). The decrease in inhibitory efficiency toward the end of the series in preparation 3 was probably due to some local impairment at the point of stimulation in the afferent nerve (cf. 23, fig. 1).

Reference to figure 1 (23) will show that since it required 40 Z units applied to the sciatic nerve to produce a maximal action current, and even though the lines of current are more concentrated in a smaller nerve such as the peroneal, the difference is not large, it is probable that none of the inhibitory stimuli shown in the table sufficed to excite

all the afferent fibers. Moreover in these experiments only the peroneal branch of the sciatic nerve was used, and it has been shown by Camis (32) that no one branch of the sciatic nerve can produce as strong a central flexion effect as can be produced by stimulation of the entire sciatic nerve.

These considerations seem to justify the way out of the difficulty which is proposed in the final chapter of Lucas's monograph (17, p. 99), namely, that a failure of complete inhibition in this center can only occur when a considerable number of afferent fibers remains unexcited and therefore a considerable proportion of the motor neurones remains free from the inhibitory effect.

An objection might be raised to the validity of these comparisons on the ground that Z units are not absolute but only relative units of intensity; that is, the stimulating value of the Z unit varies with the resistance in the secondary circuit. Under perfectly constant conditions, that is, with electrodes undisturbed in contact with a given tissue so that the secondary resistance cannot vary appreciably, Z units offer a fairly accurate standard of comparison between different successive stimuli, the error amounting only to about ± 2 per cent. In the comparison of stimuli applied to different types of tissue through different types of electrodes the variation in the stimulating efficiency is so great that the Z unit is of comparatively little value, but when stimulating electrodes of the same design and dimensions are applied in the same way to nerves of approximately the same size the conditions are so closely similar that the error in comparing the stimuli in successive experiments in Z units is probably not much greater than that in comparing successive stimuli in the same experiment. Porter (33) in a series of 52 determinations of the threshold of the nerve-muscle preparation in the spinal cat, each in a separate animal, with electrodes applied to the radial nerve, found only 21, or 40 per cent, differing from the average value by a ratio of more than two to one. Considering the possibility of real variation in irritability from animal to animal, and especially of local impairment of the nerve at the point stimulated due to handling, etc., this series may be considered good evidence of the approximate validity of the Z unit as a comparison for stimuli under similar conditions. The evidence shown in figure 1 of a previous paper (23) indicates that in appraising the real value of a stimulus far more error is likely to arise through local impairment of the nerve trunk, which usually occurs near the point at which the ligature is applied, than from differences in resistance in the secondary circuit or in the concentration of lines of

current flow when electrodes of the same design are applied in the same manner to either the peroneal or popliteal nerve in the cat (these nerves being approximately the same size).

It may probably be safely concluded that if with the electrodes applied as in these experiments maximal stimulation is only attained at about 40 Z units in the sciatic nerve, stimuli of 15 Z units or less similarly applied to either popliteal or peroneal nerve will almost certainly be submaximal; that is, will fail to stimulate all fibers. Thus we may reasonably dispose of the obstacle which Adrian recognizes as standing in the way of accepting the proposed explanation of central inhibition.

Electrical reversal. In Lucas's monograph significant facts are mentioned in support of this explanation of inhibition (17, p. 96). One is the reversal of reflex effect shown by Sherrington and Sowton to result from changing the character of electrical stimulation, already mentioned in the introduction; this may conveniently be designated "electrical reversal." The observed facts are that in the case of the extensor center faradization of the afferent nerve in the same limb, unless very weak, produces inhibition. Weak galvanic currents each lasting 0.04 second and succeeding each other at a rate of 12 per second, caused reflex excitation; currents of the same duration and frequency but approximately ten times as strong, caused reflex inhibition. Alternating currents of 20 cycles a second from a rheonome caused reflex excitation. Tiedermann is cited as showing that in a frog under strychnine, reflex excitation is converted into inhibition by an increase in the frequency of stimulation.

Lucas and Adrian (17, p. 96), (30) point out how the proposed explanation harmonizes with these facts, for in the so-called Wedensky inhibition which furnishes the model, an increase in frequency at the appropriate strength of stimulus, or an increase in strength at the appropriate frequency, would serve to convert excitation into inhibition. Further support is found in the fact that Tiedermann, and also Sherrington and Sowton, obtained with certain strengths and frequencies of faradization an initial twitch followed by inhibition (3, fig. 3), just as is found in the Wedensky inhibition.

Thus far the facts of electrical reversal fit the proposed explanation as well as could be desired. The implication is that when a series of stimuli applied to an afferent nerve causes excitation in the extensor motor neurones each impulse in an afferent fiber sets up a single impulse in the "pre-motor" neurone, and that when the frequency of afferent stimulation is increased by the required amount to produce cen-

tral inhibition, still each afferent impulse sets up a single impulse in the pre-motor neurone, but that now each comes nearly enough in the relative refractory period of this neurone to make the impulses subnormal by the amount required for their extinction at the final synapse. The application of the theory in this form to the observations of Sherrington and Sowton would require that the relative refractory period in the pre-motor neurone should last more than a 28th of a second, since inhibition is found with induction shocks of moderate intensity delivered at a frequency of 28 a second. The strengths of these shocks are not definitely given nor the primary current producing them, but from the coil distances it may be inferred that the shocks were probably not strong enough to produce compound stimulation. In my own observations strong inhibition was produced, as already stated, by induction shocks of only 10 Z units at frequencies between 40 and 50 per second. If the theory is to be applied without further complication it will require that the pre-motor neurone should still be only capable of conducting subnormal impulses, 0.02 second in my experiments, and 0.036 second in the experiment of Sherrington and Sowton, after the passage of a previous impulse. This would imply a refractory period far longer than is found in peripheral nerves in mammals at body temperature. However, this is not in itself necessarily a serious obstacle.

Greater difficulty is presented by the fact that Porter (33) has in a large number of spinal preparations evoked the flexion reflex by a single induction shock which varied in the different experiments from a minimum of less than 1 Z unit to a maximum of 21 Z units. These stimuli are presumably far too weak to produce compound stimulation in the afferent nerve. Sherrington has found that the flexion reflex regularly includes inhibition of the extensor muscles (1, p. 93), and if this can be evoked by a single induction shock too weak to produce compound stimulation, we cannot depend on any frequency of impulses in the afferent fibers for the causation of inhibition. If this is the case the application of the theory must become more complicated.

The question whether it is possible to produce reflex inhibition of a skeletal muscle by applying to an afferent nerve a single induction shock too weak to cause compound (rhythmic) stimulation, seemed to me crucial in deciding whether we may explain the phenomenon of electrical reversal in the direct and simple manner proposed by Lucas and Adrian. I have therefore made a series of experiments in a decerebrate cat to test the point. The transection was made at the anterior colliculi. The right leg was prepared for recording the contraction of the

vasto-crureus muscle as described earlier in this paper, the stimuli being applied to the popliteal nerve. After a series of records was obtained, the left gastrocnemius muscle was similarly studied, and finally the left vasto-crureus, the stimuli being applied in each case to the peroneal nerve. In the series with the left vasto-crureus the psoas muscle was not cut and only the saphenous and sartorius branches of the anterior crural nerve, since knee flexion must signify inhibition irrespective of the action of the hip flexors. The stimuli used were single break shocks, and the primary circuit was broken by the key described in an earlier paper, consisting of a sharp amalgamated copper point dipping into mercury. This key insures a clean break without risk of closure after the circuit is once broken.

In each of the three nerve centers examined in this animal, inhibition could be obtained with single induction shocks of moderate intensity. In the first test with the right vasto-crureus muscle the tonus was comparatively weak and the inhibitory effect did not regularly appear in a pure form. Sometimes it was complicated by an excitatory response and sometimes completely replaced by it. In the left gastrocnemius preparation inhibition was regularly obtained with single induction shocks varying from 5 to 80 Z units. In the left vasto-crureus center inhibition was regularly obtained with single shocks varying from 4 Z units to over 300. The inhibitory effect was rendered more marked by passive extension of the knee, before each test, the knee manifesting the "shortening reaction" (34); that is, remaining in a slightly more extended condition after passive extension than before. Figure 4 A shows a series of consecutive tests with increasing strength of induction shocks. Figure 4 B shows a similar series of tests taken immediately after those in figure 4 A with stimuli of progressively decreasing strength. In each case the drum was stopped, the knee passively extended, the drum then started, the stimulus applied, and after the reaction had been completed the drum was stopped again and the knee was again extended with the drum stationary, thus making between each two successive observations an ordinate on the drum. The entire series shown in figure 4 occupied somewhat less than 10 minutes; that is, the observations were made at a rate of between 2 and 3 a minute.

One of the afferent nerves used in this experiment was subsequently cut centrally and connected with a string galvanometer without disturbing the contacts of the stimulating electrodes. The galvanometer was of the Hindle type with a string of only 1.5μ diameter, and therefore adapted to responding rapidly to very small changes. Stimuli of

varying strengths were applied and the action currents were recorded monophasically. The responses became maximal when the strength of the break shocks had been raised to about 45 Z units; even with break shocks of more than 200 Z units no evidence could be found of a second action current in response to a single shock.

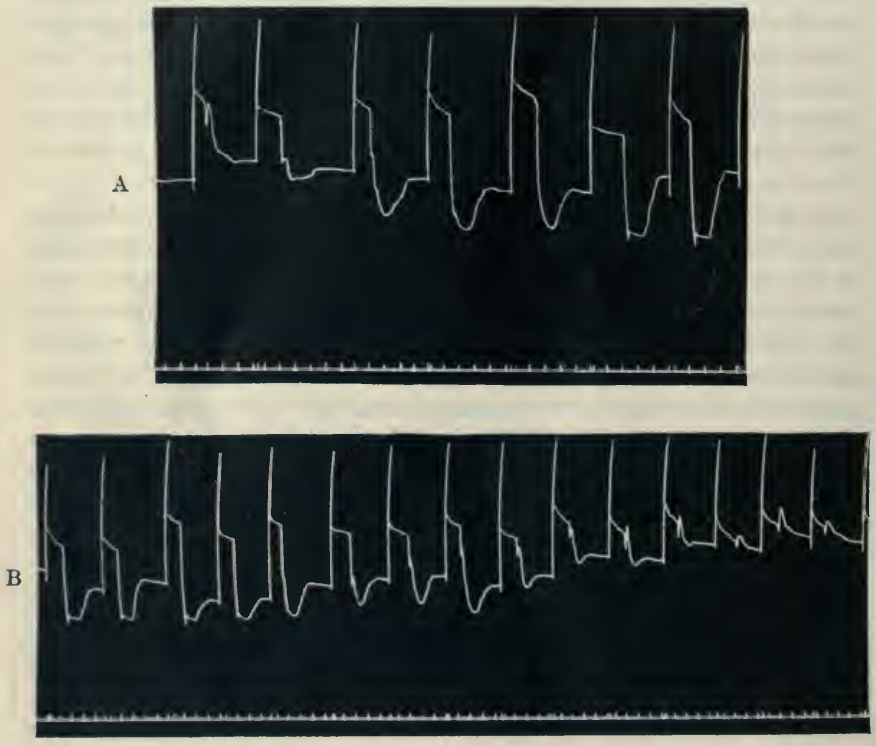

Fig. 4. Left vasto-crureus inhibited by single ascending break shocks applied to left peroneal nerve (see text). The stimuli taken in order were as follows: in A, 18 Z, 27 Z, 33 Z, 42 Z, 54 Z, 73 Z, 105 Z; in B, 149 Z, 105 Z, 73 Z, 54 Z, 42 Z, 33 Z, 27 Z, 22 Z, 18 Z, 13 Z, 10 Z, 8 Z, 6 Z, 5 Z, 4 Z. Speed of drum constant throughout. The records in A are photographed on a larger scale than in B. Time in seconds below.

Clearly inhibition can be caused by a single induction shock almost certainly too weak to set up more than one impulse in a single fiber; therefore the electrical reversal cannot be explained by the simple extension of the stimulation frequency into the pre-motor neurone. Inhibition must depend on something other than the peripheral stimu-

lation frequency. This does not mean that it cannot depend on a special frequency of impulses in the premotor neurone, as proposed by Lucas, but that if this frequency exists it must be set up in some other way. It may be that convergence of many afferent fibers at a single internuncial neurone will suffice to impart to it the necessary frequency of excitation. The conduction times of the different fibers converging on the synapse might differ enough to provide a rapid sequence of separate impulses arriving at the confluence of the dendrites. This explanation would imply a very high frequency, since the total reduced reflex time in the flexion reflex is about 4σ (35, p. 144), and it is unlikely that the conduction times of different afferent paths to the pre-motor neurone would differ from each other by more than a small fraction of this amount. It may be significant in this connection that Gregg and I found reason to infer that certain central portions of reflex conducting paths have briefer refractory periods than the afferent nerve fibers (23, p. 221).

It is quite conceivable that the change from inhibition to excitation on reduction of the strength of afferent stimulation might be explained on this same basis of converging afferent fibers. If the stimuli are reduced to submaximal strength fewer fibers would be stimulated, and consequently there might be fewer impulses set up by the different converging fibers in a given time; the frequency in the pre-motor neurone might thus be reduced below the critical value and thus become excitatory. Also the current of gradual onset from the rheonome, by initiating the individual impulses at different instants instead of simultaneously, might favor a lower frequency of arrival at the synapse on the part of the converging impulses. Thus through the principle of convergence we may arrive at a possible explanation of electrical reversal.

We must not forget that tonus has been found a prerequisite for this excitatory effect. Sherrington and Sowton found the ipselateral extensor contraction unobtainable in the decapitate preparation (3, p. 442), and in the decerebrate animal either absent or poor unless the reflex tonus of the muscle was good. This fact in itself shows that the excitatory effect cannot be conditioned solely by an appropriately low frequency of afferent stimuli; there must be a certain reinforcement through other central paths. How to fit such reinforcement into the scheme of critical frequencies in an internuncial neurone is difficult to see, although a possible method may be found with the aid of additional nervous connections for whose existence reasons will be shown presently.

Rebound. At this point we should consider the question of extensor rebound contraction following inhibition. An early view of this response was that the reflex energy of which tonus was the expression accumulates during inhibition, and upon its release breaks through with renewed intensity. Aside from the fact that in this way of looking at rebound we are treating tonus as if it were a stream of water, and ignoring the nature, mode of initiation and of propagation of the nerve impulses which make up reflex activity, the view is untenable for reasons which I have discussed elsewhere (6, p. 160). Sherrington found that the amount of rebound contraction following inhibition shows no correlation with the amount of tonic activity inhibited (4, p. 59); for instance, inhibition prolonged beyond a certain time is followed by less rebound than if the inhibition is briefer. Rebound therefore cannot depend merely on the accumulation of the suppressed tonus. Neither can it depend solely on the flexed position of the limb, for if so, the duration of inhibition (flexion) should make no difference; furthermore rebound occurs in the de-afferented preparation (14, p. 394), which would be impossible if it depended on proprioceptive impulses from the inhibited muscle. Apparently rebound depends on some central effect set up by the inhibitory stimulus itself, an effect which fatigues if this stimulus be continued long enough.

Looking at rebound from the point of view of nerve impulse frequency an attractive hypothesis for explaining it would be to suppose that during the afferent stimulation which produces inhibition the impulses converge at the pre-motor neurone with such frequency that the inhibitory rhythm is set up in it, but that on cessation of stimulation the frequency of impulses impinging on this neurone decreases till the rhythm in it falls below the critical value and becomes excitatory. This is quite conceivably so, but it requires some further assumption to account for the persistence of a series of impulses impinging on the pre-motor neurone for several seconds after the stimulation of the afferent nerve has ceased. Some central mechanism must be excited which continues to transmit impulses to the motor neurones for a long time after the afferent impulses have ceased to come in. We may possibly account for this fact without postulating in the central complex any properties foreign to peripheral conducting paths, if we assume a sufficiently extensive series of branching paths in which conduction is delayed, as it probably is in the synapses. A single impulse might, through extensive branching of the fiber which conducts it, set up other impulses in a large number of central neurones, and these in turn might

set up impulses in other neurones, and the synaptic delays in some of the more extensive paths might suffice to account for the observed continuance of the motor response through many seconds; and yet there need not exist in each conducting path any functional property which has not been demonstrated in the nerve-muscle preparation.

The same considerations apply to the crossed extension reflex, in which the after-discharge is perhaps its most striking feature. Sherrington has shown a crossed extension reflex with an after-discharge of more than 12 seconds (1, p. 77). This is probably in part due to the secondary reflex effect originating in the proprioceptive impulses coming from the extensor muscle itself. Sherrington has shown that the "shortening reaction" is attributable to these afferent impulses (34), and the after-discharge of the crossed extension reflex is undoubtedly to some extent a kindred phenomenon. On the other hand a considerable after-discharge can occur when all these proprioceptive impulses are completely cut off, as is shown by the after-discharge amounting to several seconds occurring in the crossed extension reflex in the de-afferent preparation (6, fig. 16).

Additional instances of delayed reflex response which may be likened to the after-discharge in the case of the crossed extension reflex are shown in figure 4 B and figure 5, the latter being obtained from the right vasto-crureus preparation in the same animal whose left vasto-crureus furnished the records in figure 4. In each case induction shocks of moderate intensity, and therefore almost certainly setting up only a synchronous volley of single impulses in the afferent nerve, provoked complex central responses involving alternations between flexion and extension; i.e., between excitation and inhibition (cf. 14, p. 372). They illustrate the initiation of complex central effects far outlasting the arrival of the afferent impulses in the central structures, and therefore demanding for their execution far more than simple unbranched conduction to the pre-motor neurone. In this connection we should recall the observation of Dreyer and Sherrington (36) which led them to conclude that with single induction shocks of moderate intensity, "repetitive volleys" of impulses are evoked in the motor neurones involved in the simple flexion reflex. The triphasic responses appearing in figures 5 and 6, beginning with excitation, changing into inhibition, and ending with excitation, might be explained if we suppose the initial contraction to be due to the first full-sized impulse set up in each pre-motor neurone before the inhibitory frequency has been established and the final contraction to be caused by a decrease in frequency such as was suggested to explain rebound.

From the various facts enumerated above we must conclude that to accept the proposed explanation of inhibition we must recognize that the frequency of impulses in the pre-motor neurone does not correspond

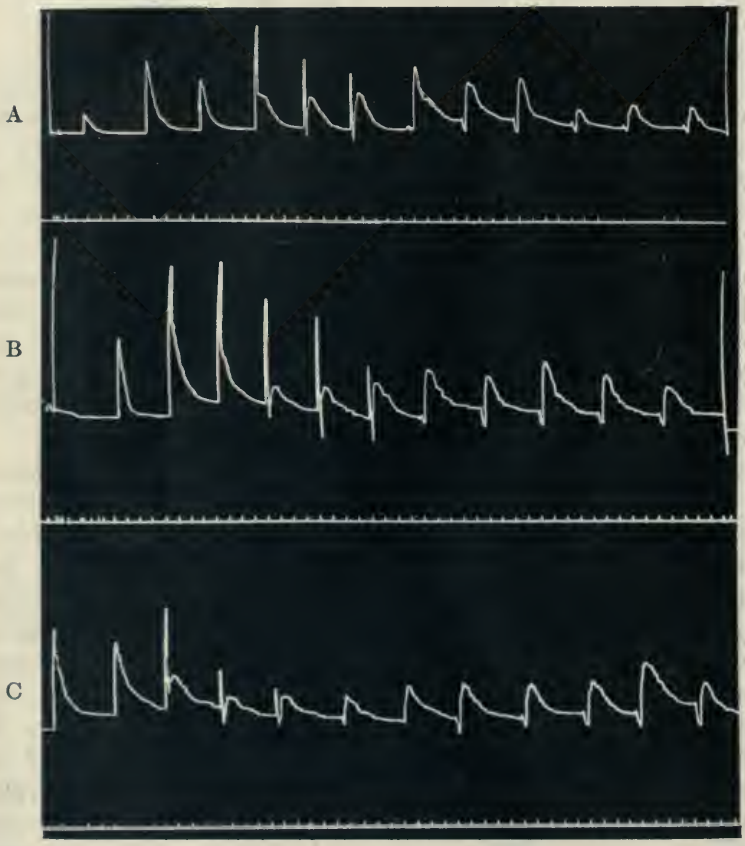

Fig. 5. Right vasto-crureus muscle. Single ascending break-shocks applied to right popliteal nerve. In each series the drum was not stopped between observations. The secondary coil distance was decreased after each stimulus. Stimuli in Z units as follows: A and C, 46, 59, 81, 116, 164, 240, 319, 408, 484, 545, 593, 631; B, 36, 46, 59, 81, 116, 164, 240, 319, 408, 484, 545. Time in seconds below.

directly with the frequency of impulses in the individual afferent fibers, and may indeed be only very indirectly dependent on that frequency (a condition which may be fulfilled by convergence of afferent fibers at a common central neurone); and furthermore, afferent impulses must in

some cases excite in the center some mechanism which enables the motor response to outlast the influx of afferent impulses far more than could be explained simply by their direct convergence upon the pre-motor neurone. There must be more elaborate paths than the simple termination of afferent fibers at the pre-motor neurone to account for the various delayed effects, but such paths need not preclude the presence of direct connection as well.

Chloroform and ether reversal. We must now consider the conversion of reflex excitation into reflex inhibition under the influence of chloroform, described by Sherrington and Sowton, and with it the transitional phenomenon under the influence of light ether anesthesia which forms the primary subject of this paper. In the final chapter of Lucas's monograph it has been suggested that this effect of chloroform may be readily explained by its known action in producing in a peripheral nerve a decrement in conduction. It is well known that nerve centers are more sensitive to the action of chloroform than nerve trunks, and therefore it is quite reasonable to suppose that if the synapse is a region of decrement, then at an early stage in the action of chloroform this decrement would be increased to the point of extinguishing those impulses which were previously able to pass the final synapse. Impulses traversing the pre-motor neurone at a frequency such that, in absence of chloroform, each was large enough to traverse the final synapse and excite the motor neurone, might fail to do so when the decrement at this final synapse had been increased by the action of the drug. In this way excitation might be replaced by inhibition.

Next comes the question whether this explanation will cover the facts shown in this paper; namely, the partial suppression under ether of the crossed extension reflex during the application of the stimulus, followed by a marked increase in extensor contraction as soon as stimulation ceases. There is no reason to suppose that ether will increase the decrement in every synapse in a spinal center by precisely the same amount. Let us suppose, therefore, that in some of the motor neurones the decrement is increased enough to make the existing frequency of nerve impulses in the pre-motor neurone inhibitory, but that in other motor neurones the effect has not sufficed to make this change. Under this condition we shall get during the application of the stimuli inhibition in some neurones and excitation in others, and consequently the observed intermediate degree of contraction in the muscle.

But this explanation alone will not account for the increased contraction following cessation of the stimuli. We have still to explain

the fact that some of those motor neurones which are inhibited during afferent stimulation are excited when it ceases. The crossed extension reflex is normally characterized by a prolonged after-discharge, and we have already found it necessary to postulate some elaborate conducting paths involved in this reflex arc. In this way we have established a condition for the continuance of motor response after afferent stimulation has ceased, but we have yet to find a mechanism for the sudden replacement of inhibition by excitation. It is possible that we may invoke the explanation proposed for rebound contraction, namely, that the impulses converging at the individual pre-motor neurone during afferent stimulation arrive with an inhibitory frequency, but that after stimulation has ceased they arrive less and less frequently. Some arcs with an increased decrement at the final synapse would then become inhibitory while impulses were crowding in rapidly as a result of afferent stimulation, but would still have a small enough decrement for the impulses at the reduced frequency (after afferent stimulation has ceased) to pass through. This view simply applies the principle of convergence already proposed to explain rebound in the homolateral arc, to the terminal effect in the more complex arc involved in the crossed extension reflex with its characteristically long delay. But it requires that the impulses from the crossed arc shall arrive at a given pre-motor neurone with a lower frequency than in the case of the homolateral arc, since in absence of narcosis the normal response even to the most powerful afferent stimulation is excitation instead of inhibition.

Just as the narcotic reversal may be explained on the basis of increased decrement in the final synapse, so the opposite reversal by strychnine (9), (37) (conversion of inhibition into excitation) might be explained on the basis of diminution of decrement. If the decrement in these synapses largely disappears, a frequency of impulses in the pre-motor neurone, normally inhibitory, would become excitatory.

It should be mentioned that Bayliss (38) has found effects of chloroform and strychnine on the vasomotor reflexes quite similar to those which Sherrington and his co-workers have found in the case of the limb reflexes, and it is probable that the ultimate explanation of one group of phenomena will hold good, at least in part, for the other.

Reciprocal innervation. Thus far the discussion has dealt wholly with the extensor motor neurones. In any working scheme we must allow for the reciprocal effects in the flexor motor neurones. The same stimulus which inhibits the extensors regularly excites the flexors (1, p. 93); the afferent fibers through which we excite the flexor motor neu-

rones presumably give off branches which inhibit the extensors. This mechanism may be harmonized with the proposed scheme by assuming that the convergence of paths at individual pre-motor neurones in the flexor center is such that a lower frequency results in them from the simultaneous excitation of the afferent fibers than in the extensor center. Or the same result may be obtained if the decrement in the final synapse in the flexion reflex arc is less than in the corresponding synapse of the extensor arc, so that a frequency which in the pre-motor neurones of the extensor center is inhibitory, will in those of the flexor center be below the critical frequency, and therefore excitatory. That even the simple flexion reflex, evoked by a single induction shock, involves more than one impulse in each motor neurone, is suggested by the observations of Dreyer and Sherrington (36), although Gregg and I (35) were unable to find evidence to substantiate this view, except in the case of induction shocks so strong as to produce compound stimulation (23).[1] If the reflex involves only a single impulse in each pre-motor neurone, the question of frequency does not arise, since this neurone, if not already occupied, will then conduct a full-sized impulse which will readily pass through the final synapse and excite the motor neurone.

The inhibition of flexion during the crossed extension reflex could conceivably depend on convergence of fibers at the individual pre-motor neurone in the flexor center, imparting to it an inhibitory frequency of impulses. This presents a difficulty due to the fact that maximal afferent stimulation produces a flexion reflex which cannot be inhibited by any contralateral stimulus. Reflex inhibition which in the extensor center under maximal ipselateral stimulation constitutes an absolute block, fails in the case of contralateral flexors to block the reciprocal excitatory effects of strong ipselateral stimulation. According to the proposed explanation by frequency, a block should be absolute; no amount of stimulation of other nerves should break through it. In a later section we shall find reason to assume additional connections which will provide a different explanation of the inhibition of contralateral flexors, and thus offer a way out of the difficulty.

Postural reversal. We now come to the postural reversal or "Umkehr," which has been described by Sherrington (39, p. 299) and also studied by Magnus (40). In general this means the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied. In the old-fashioned terminology this

[1] cf. Sassa and Sherrington: Proc. Roy. Soc., 1921, xcii, 108.

is described as a "tilting of the neural balance." Sherrington has found that certain stimuli will cause flexion if the limb is already passively extended, and extension if it is flexed. Magnus has found that the cat's tail when stimulated at the tip is reflexly drawn toward the median plane from whichever side it happens to be hanging. He has shown that this phenomenon depends upon proprioceptive impulses; that is, afferent impulses coming from receptors within the muscle. Since passive flexion stretches the extensor muscles and favors reflex extension, it is supposed that the afferent impulses from a stretched muscle predispose to an excitatory response the motor neurones of that muscle, and this view is supported by the above-mentioned observation of Magnus. Graham Brown (41) has found in the guinea pig a reversal in the opposite sense, due to actively maintained posture; when the limb was actively in a state of flexion the reflex response was further flexion, and when it was actively extended the reflex response was further extension. The two facts might be reconciled as both due to increased state of tension within the muscle, since either passive flexion or active extension will increase the tension in the extensor muscle. On the other hand I have obtained evidence of postural reversal in the same sense as that shown by Sherrington and Magnus even in the case of actively maintained posture. This is shown in the comparison between figures 5 and 6. Figure 6 was from the same animal, and made after the observations shown in figure 5 B and immediately before those in figure 5 C. In figure 5 each stimulus was applied after the limb had returned to a semi-flexed posture following the preceding stimulus. In figure 6 two observations are shown, in each of which the crossed extension reflex was evoked by pinching the toe of the opposite foot; during the subsidence of this response and while the limb was still considerably extended, a single shock was applied (as in fig. 5) to the ipselateral nerve. In figure 6 A the stimulus was of the same strength as in the third observation of figure 5 C. In figure 6 B the stimulus was the

A B

Fig. 6. Right vasto-crureus muscle (see text). Stimuli, single break shocks: A, 81 Z; B, 59 Z.

same as in the second observation of figure 5 C; it was applied just after the second peak in the myogram, the response to the stimulus of pinching being in itself rhythmic. In every one of the four observations under comparison the response is triphasic, first excitatory, then inhibitory, then excitatory. In each case when the stimulus was sent in during the state of reflex extension the inhibitory part of the response was very much increased in magnitude. This fact argues against harmonizing the reversal of Graham Brown with that of Magnus and Sherrington on the common ground of tension, for presumably the tension is greater during this state of active contraction than in the less active state, although it may be less in certain idle fibers, and it is conceivable that these idle fibers form a majority of the whole muscle and that their lack of tension is what determines the character of the response. In the experiment shown in figure 6 the motor nerves to the flexor muscles were cut, and therefore any proprioceptive afferent impulses influencing the result must have come from the extensor muscle itself.

In opposition to the view that the tension irrespective of the degree of shortening is what determines the nature of the afferent effect on the central response, may be cited the observations of Sherrington on "plastic tonus" (34), (42). He described what he calls the "shortening reaction" and the "lengthening reaction" in the vasto-crureus preparation of the decerebrate cat with moderate degrees of decerebrate rigidity. In the "shortening reaction" when the extensor muscle is allowed to shorten by passive extension of the knee it "takes up slack" and remains shortened; in the "lengthening reaction" when the knee is forcibly flexed there is inhibition of decerebrate tonus in the muscle and the knee remains flexed. Sherrington showed that these responses are reflex in nature, depending on afferent impulses arising in the vasto-crureus muscle itself. He further showed that in the case of the "shortening reaction" essentially the same thing happened if the muscle was reflexly contracted, and that in either case it was the shortening of the muscle—it made no difference whether actively or passively produced —that provided the adequate stimulus for the central effect. In short, the degree of shortening, irrespective of tension, seems to be the condition that determines what afferent impulses are set up. This fact suggests the view that the predisposing influence on the central response which Magnus and Sherrington have found traceable to posture, probably depends on the effect of actual shortening or lengthening of the muscle rather than on the amount of tension. This view would har-

monize the observations shown in figure 6 with the observations of Sherrington and Magnus.

The reversal in the guinea pig described by Graham Brown seems to be at variance with this principle. It should be recalled that this reversal was most marked in the intact animal in which the cerebral cortex was free to modify reflex behavior, and that it was only with difficulty obtained in the decerebrate animal. Moreover, the stimulus used to evoke the reflexes, manipulation of the "femoral fold" of the skin, was not very well adapted to qualitative regulation or to accurate limitation of the stimulus to the same fibers in every test. The apparent contradiction may be attributable to these considerations, or it may be due to a difference in the organization of the guinea pig's centers from those of the cat, with respect to postural regulation.

The explanation of the divergence, proposed by Graham Brown (41, p. 287), does not harmonize well with the knowledge of the nerve impulse which has come to light since his paper was published. He suggests that the actively maintained posture is an expression of a heightened state of excitation in the center of the tonically contracted muscle, and that to this excitation is added that induced by the sensory stimulus. We now know that a nerve impulse cannot be increased in magnitude by any increase in the stimulus which initiates it. On the other hand, such an effect as he invokes might under certain conditions be simulated by a summation of propagated disturbances such as Adrian and Lucas have described (25).

In fitting these facts of reversal into our proposed scheme we must remember that in general increased convergence of impulses at a pre-motor neurone decreases the chance of excitation of the motor neurones and increases the chance of inhibition. Let us assume that shortening of the extensor muscle, active or passive, sets up afferent impulses of a certain frequency, and that these set up impulses in the pre-motor neurones of the extensor center; we have then the condition for the "shortening reaction." Following our proposed scheme, the resulting frequency in the pre-motor neurones must be too low to be inhibitory. Those afferent stimuli whose central effect is subject to postural reversal may in themselves deliver to the pre-motor neurones in the extensor center impulses of a frequency also below the critical value. If when this stimulus is applied the limb is in a flexed posture and no impulses are coming to the pre-motor neurones through the afferent fibers from the extensor muscle itself, then the stimulus will cause an excitatory response, resulting in extension. But if the limb is already extended,

with the result that impulses of an excitatory frequency are already being set up in the pre-motor neurones by the afferent impulses from the muscle, and then if the external stimulus causes additional impulses to impinge on these pre-motor neurones, we shall have the condition for inhibition, because their effect will be to increase the frequency of impulses in these neurones above the critical value. Flexion will therefore result. In this way the proposed scheme might provide a mechanism for the "Umkehr" or postural reversal of reflex effect.

Before leaving the subject of postural effects we should note that the histologists have found two distinct types of sensory nerve endings in skeletal muscles,—free nerve endings and those which terminate in the structure of the muscle spindle (43). By assigning different functions to these two types we might find a further means of explaining inharmonious observations, but we should be adding to the complexity of a picture which has already been complicated beyond the indications of experimental fact.

Intrinsic alternation of flexion and extension. One more phenomenon of fundamental importance in the behavior of nerve centers remains to be considered,—the tendency to rhythmic alternation between flexion and extension even after all afferent impulses have been eliminated. Sherrington (44) has shown that the scratch reflex proceeds with unaltered rhythm in response to appropriate cutaneous stimulation, even after severance of all afferent fibers coming from the muscles involved. More recently Graham Brown (16) has studied the rhythmic alternation between flexion and extension which is characteristic of progression. This rhythm is slower than that of the scratch reflex, being usually one to two beats a second. He found that the intact animal under general narcosis tends to exhibit these movements spontaneously. At a moderate depth of narcosis transection of the spinal cord in the lumbar region will interrupt them. Their independence of afferent impulses is shown by their persistence after the severance of all afferent fibers from the limb under observation, and especially by their persistence at a depth of narcosis at which neither strong stimulation of a nerve in the opposite leg nor transection of the spinal cord produces any response. The progression rhythm persists under increasing narcosis almost if not quite as long as respiration. The rebound contraction disappears much earlier in narcosis than the progression rhythm.

Graham Brown proposes as an explanation (16, p. 37) that the motor neurone sends off a branch-fiber through which it may inhibit activity in the motor neurones of the antagonistic muscle. He mentions mor-

phological evidence of a branch leaving the axone of the spinal motor neurone not far from the cell body. To account for the rhythmic alternation he suggests that this inhibitory effect may become fatigued and thus in time enable the inhibited neurone to become active and in turn inhibit its antagonist.

Let us see how this phenomenon can be treated in accordance with our scheme of analysis. If we assume that the collateral branch of the motor neurone leads to the pre-motor neurone of the antagonistic center

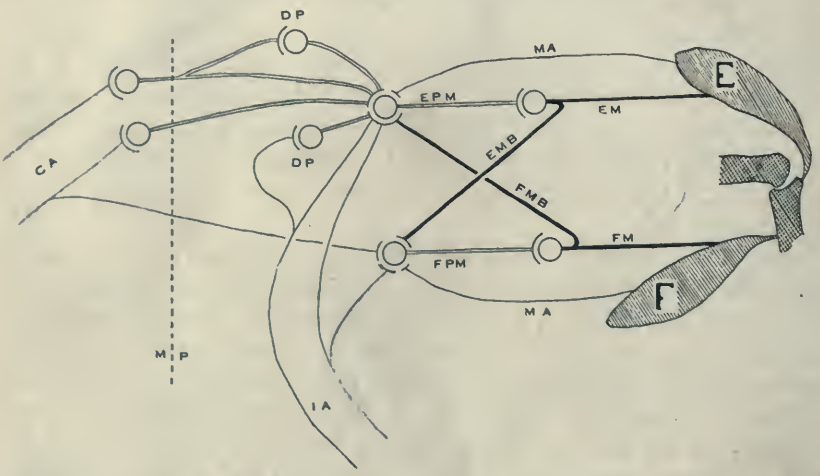

Fig. 7. Diagram of spinal neurones and their connections to provide a possible basis for analysis of reflexes. Afferent neurones shown in light lines; internuncial neurones in double lines; motor neurones in heavy lines. *E*, extensor muscle; *F*, flexor muscle; *M P*, median plane; *C A*, contralateral afferent fibers; *I A*, ipselateral afferent fibers; *D P*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *E P M*, extensor pre-motor neurone; *F P M*, flexor pre-motor neurone; *E M*, extensor motor neurone; *F M*, flexor motor neurone; *E M B*, collateral branch of extensor motor neurone; *F M B*, collateral branch of flexor motor neurone.

(fig. 7) we shall at once establish the condition for converting excitation into inhibition in that center. Thus if a pre-motor neurone in the extensor center is at a given moment conducting impulses with a frequency below the critical value, and therefore causing excitation in the motor neurone, then if a series of impulses is set up in the flexor motor neurone, these will by virtue of the collateral branch leading to the extensor pre-motor neurone, set up in it additional impulses which will raise the frequency therein above the critical value, and inhibition will

result. The same picture may be reciprocally applied to the inhibition of flexors through activity in the extensor motor neurones. But the question at once arises, if impulses in the extensor motor neurone suffice to set up in the flexor pre-motor neurone impulses which combined with those from another source will be of inhibitory frequency, how can a flexion reflex be produced by afferent stimulation? In point of fact, we know that moderate stimulation of an appropriate nerve will inhibit the strongest extensor contraction and produce a flexion reflex. This may be explained as the result of the direct convergence of the afferent fibers at the extensor pre-motor neurone producing in it an inhibitory frequency in the manner already discussed. The resulting inhibition of the extensor motor neurone would stop the stream of impulses in the branch leading to the flexor pre-motor neurone and leave the latter free to be effectively excited through the afferent path. We may also invoke the aid of synaptic fatigue, a condition closely paralleled in the nerve-muscle junction and actually demonstrated in certain reflexes (29). The synapse at which the collateral branch of the extensor motor neurone makes contact with the flexor pre-motor neurone may fatigue rapidly and become non-conducting. If this were so then the inhibitory effect on the flexor center of impulses in the branch of the extensor motor neurone would soon wear off and the flexor pre-motor neurone would be free to respond to an excitatory stimulus without being first released in the roundabout way outlined above. Such a synaptic fatigue seems necessary to provide for the intrinsic progression rhythm described by Graham Brown as occurring in absence of rhythmic afferent stimulation.

The connections described will with the aid of synaptic fatigue explain the rhythmic alternation between flexion and extension, provided there is some initial source of nerve impulses in the central (pre-motor) neurones. Indeed some such source must exist no matter how we organize the neurones or what connections we provide. The scheme outlined provides for the intermittent interruption of impulses first in one set of motor neurones and then in the other, but what is to start them again? Either we must assume something in the grey matter of the spinal cord which can initiate nerve impulses, something possibly excitable by a so-called blood stimulus, or we must assume that through some such elaboration of branching paths as was suggested to explain after-discharge, a small disturbance, once started, is kept alive and enabled to excite a neurone again after the stage of inhibition is past.

It is possible that we might greatly simplify the entire scheme by relegating most of the inhibitory effects we have examined to the collateral branches of the motor neurones which Graham Brown has postulated for the explanation of progression rhythm. If we could explain the reciprocal innervation of antagonistic muscles as due to the effect of these branches in raising the impulse frequency in the antagonistic pre-motor neurone above the critical value, and thus make inhibition of one center secondary to the excitation of motor neurones in the opposite center, we should avoid the need of the somewhat difficult assumption made at the start, that the convergence at the individual pre-motor neurones of afferent fibers with different conducting times is responsible for imparting to them the inhibitory frequency. By invoking the aid of these branches we should make the inhibitory frequency depend on the arrival of impulses from them at the pre-motor neurones in alternation with those coming directly through the afferent fibers. In particular, this explanation would clear away the difficulty we met in trying to explain crossed (contralateral) inhibition of flexors by convergence of afferent fibers, for if inhibition of the flexors depends on joint action through the opposite afferent nerve (fig. 7, *C A*) and the collateral branch, *E M B*, of the extensor motor neurone, then all that is necessary to release the flexor motor neurones from inhibition is to stop the discharge of impulses in the extensor motor neurones. But here we must introduce a difference between flexors and extensors to account for the dominance of the flexion reflex. It must be possible to inhibit the extensor center without first initiating impulses in the flexor motor neurones; otherwise it would be impossible to break through the block of inhibition in the flexor center, as is done by a powerful ipselateral stimulus. To this end we must retain the idea of inhibition of extensors by convergence of afferent fibers, *I A*; but the crossed inhibition of flexors may well depend on the combined frequency of impulses arriving through afferent fibers and through collateral branches of extensor motor neurones.

There is another argument for this differentiation between the mechanisms of flexor and extensor inhibition. We have seen that the progression rhythm in absence of afferent impulses requires rapid fatigue in the synapse at which the collateral branch approaches the opposed premotor neurone. But if inhibition always depended on these branches and not on direct afferent convergence as at first proposed, then how could we reconcile the assumed rapid synaptic fatigue with the observed fact that strong afferent stimulation will keep decerebrate rigidity in

the extensors inhibited for a long time? This might be explained if the synaptic fatigue did not readily progress beyond a certain point at which it established a decrement sufficient to block very small impulses such as would occur if they followed each other with high frequency, but insufficient to block the larger impulses occurring at lower frequencies. The persistently dominant flexion reflex would on this view involve the slower frequency of impulses. In this connection it should be noted that in earlier experiments on an inhibitory reflex I have observed fatigue involving only the particular reflex arc employed, and probably therefore occurring in the synapse (29, p. 116). But such an explanation becomes unnecessary if we adhere to the view of afferent convergence to account for inhibition of extensors.

It will be recalled that the reinforcement by tonus of the excitatory phase of the electrical reversal, described by Sherrington and Sowton, appeared difficult to reconcile with the explanation of inhibition by the convergence of afferent fibers. This difficulty can perhaps be met with the aid of "summation of propagated disturbances," (25) enabling an afferent impulse appropriately timed after other impulses from the hind-brain to get through a synapse where the decrement is too great for a single impulse to pass.

We might attempt further analysis by assuming that the collateral branches of the flexor motor neurones are less subject to synaptic fatigue than the extensors, or that few reflexes involve all the motor neurones of a center at once, they being usually active and idle in rotation, or by assuming that in many cases when a chain of connections is traced from the flexor center through the collateral branch to the extensor center and back to the flexor center, it does not lead back to the same individual neurone from which it started, or by introducing the additional assumption that two or more pre-motor neurones may converge at a single motor neurone and thus provide a higher frequency in the collateral branch than that in any one pre-motor neurone contributing to it. But all these maneuvers are so complex and so speculative that it seems hardly worth while to follow them.

CONCLUSION

This analysis of reflex organization has led into many details which have little or no foundation either in experiment or anatomy, and which may therefore seem far-fetched and improbable. It would be simpler and perhaps more satisfactory to recognize merely two antagonistic

central effects induced by every afferent stimulus, as was done in the introduction, and to assume that these effects depend merely on the excitation of the two antagonistic mechanisms or groups of neurones each so arranged as to produce its appropriate effect on the motor neurones. Such a simple assumption would not conflict with the explanation of inhibition proposed by Lucas, for we might assume that each group of neurones in some way has the property of imparting to the pre-motor neurones of one center a relatively slow and therefore excitatory frequency of nerve impulses, and to the pre-motor neurone of the opposite center a more rapid and therefore inhibitory frequency; but such a simple assumption would fail to explain how these groups of neurones contrived to impart these determining frequencies to the pre-motor neurones. We should be leaving the analysis incomplete and describing the effect without attempting to explain the method. The more elaborate analysis as proposed furnishes a complete set of possible if not probable details for explaining all the facts considered without invoking any peculiar principle of reflex behavior or any functional capacity which has not been clearly demonstrated in the simple conducting paths constituting the nerve-muscle preparation, which have been so thoroughly analyzed in the laboratory. The proposed analysis is not set up as being in its present form at all probable. The underlying idea in carrying it as far as I have has been that expressed by Keith Lucas (17, p. 2) as follows: "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?" As a method of attacking the problem he suggests—"that we should first inquire with all care whether the elementary phenomena of conduction, as they are to be seen in the simple motor nerve and muscle, can give a satisfactory basis for the understanding of central phenomena; if they cannot, and in that case only, we shall be forced to postulate some new process peculiar to the central nervous system." Guided by this idea I have sought to examine those of the more definitely established facts of reflex behavior with which I am acquainted, with a view to seeing if the known functional capacities of the peripheral conducting tissues could be made a possible basis for explaining them all.

The result of the analysis is of course inconclusive in the present state of our knowledge; it neither proves nor disproves the applicability of Lucas's proposed explanation of inhibition to reflex phenomena. But I believe it is worth recording that the attempt has as yet brought to light no insuperable obstacles.

SUMMARY

1. The decerebrate cat under a certain degree of ether narcosis exhibits a modification of the crossed extension reflex, as follows: During the application of a strong tetanizing stimulus to an afferent nerve in the hind limb, the knee extensor muscle in the opposite hind limb exhibits instead of the usual full contraction, a partial contraction during the application of the stimulus, followed on its cessation by a distinct increase in contraction. In some preparations this increase is marked, in others only barely discernible. The effect disappears as the animal is allowed to come out of ether.

2. This effect is similar in one respect to the conversion of reflex excitation into inhibition under chloroform, described by Sherrington and Sowton; in another respect it is similar to the rebound contraction occurring in extensor muscles after reflex inhibition. Taken in connection with these observations and with the strychnine reversal of reflex effect and the electrical reversal described by Sherrington and Sowton, this observation tends to support the view that in general the stimulation of a single afferent nerve in a hind limb tends to evoke simultaneously two antagonistic reflex effects one of which usually dominates and masks the opposite effect which, though latent, is always present.

3. An attempt is made to analyze these various reflex effects in terms of the nerve impulses of which they are composed, avoiding the postulation of any properties in the central conducting path differing fundamentally from those which have been demonstrated and analyzed in the nerve-muscle preparation.

4. In view of the all-or-none law of nerve conduction the differences in the effects of different afferent stimuli must depend ultimately on differences in the number of afferent fibers stimulated and in the frequency with which each individual fiber is made to respond.

5. Certain facts concerning reflexes require for their explanation the convergence of several afferent conducting paths or their central connections, at a single motor neurone.

6. Proceeding from the basis of these facts an attempt is made to see how far the explanation of reflex inhibition as proposed by Lucas can be applied in the present state of our knowledge to some of the more definitely established facts concerning reflexes. The proposed explanation is based on the analysis by Lucas of so-called Wedensky inhibition. Briefly it is that the impulses follow each other in a conducting path with such frequency that each occurs during the relative refractory period following its predecessor and therefore being subnormal, cannot pass the region of decrement existing at the next synapse without extinction; an internuncial ("pre-motor") neurone being the last link of the chain leading to the motor neurone, has a critical frequency of nerve impulses above which their effect will be inhibitory and below which excitatory.

7. The balancing or algebraic summation of antagonistic central effects described by Sherrington is examined in the light of our present knowledge of the intensity of the afferent stimuli required to produce the effect. This evidence makes it extremely improbable that such balancing can ever be obtained in extensors with inhibitory stimuli strong enough to excite all the fibers in the afferent nerve. This phenomenon, therefore, which has hitherto been regarded as an obstacle to the application of the supposed explanation need not be so considered.

8. The electrical reversal or change from inhibition to excitation on changing the intensity and character of the electrical stimulus applied to an afferent nerve cannot be as simply explained on the basis here considered as appears at first sight. Reflex inhibition can be produced by a single induction shock of moderate intensity, too weak to set up more than a single impulse in each afferent fiber. Therefore if reflex inhibition so produced depends on the frequency of impulses in the pre-motor neurone, this frequency must be set up by convergence of afferent paths or other central connections and not depend directly on the frequency with which the afferent fibers are stimulated.

9. It is suggested that rebound contraction following inhibition may depend on a decrease in the frequency with which impulses converge at the pre-motor neurone when they cease to enter the central mechanism through the afferent fibers. Both rebound and the after-discharge of such reflexes as the crossed extension reflex require an elaboration of central conducting paths great enough to introduce a long delay in the arrival of some impulses at the motor neurones.

10. The conversion of excitation into inhibition by chloroform can be readily explained, as has already been proposed by Lucas, as resulting

from increased decrement at the synapse due to the action of the drug. The observation first described in this paper may be explained as the result of a similar action occurring in some of the individual reflex arcs, together with a change in the frequency of converging impulses such as was invoked to explain rebound.

11. The reciprocal innervation of antagonistic muscles requires appropriate branches so arranged that impulses shall converge at the pre-motor neurones with the proper frequencies to produce excitatory and inhibitory effects.

12. The postural reversal of reflex effect described by Magnus and Sherrington, lends itself well to explanation on the proposed scheme, if we may assume that the proprioceptive impulses on which the effect has been shown to depend are induced by a shortened state of the intramuscular receptor, and arriving at the pre-motor neurone, cause the excitatory frequency of impulses existing therein to be raised above the critical value and become inhibitory.

13. The intrinsic tendency of the spinal centers to exhibit a rhythmic alternation between flexion and extension in absence of afferent stimulation and at a depth of narcosis at which even transection of the spinal cord causes no effect, may be explained by further elaboration of a suggestion by Graham Brown, i.e., that collateral branches are given off whereby impulses may be conducted to the pre-motor neurone of the opposite center and there produce jointly with impulses entering through other paths an inhibitory frequency. The rhythmic alternation would require a still further assumption of a certain measure of synaptic fatigue causing this effect after a brief time to become inoperative and the excitatory frequency therefore to be resumed.

14. The proposed explanations are speculative and more complex than the simple assumption that stimulation of an afferent nerve excites in every instance two antagonistic central effects, but they serve the purpose of demonstrating that so far as we can see at present there are no insuperable obstacles in the way of explaining reflex phenomena on the basis of nerve impulse frequency proposed by Lucas.

I wish to express my thanks to Miss L. Hompe, Miss A. Hopkins and Miss M. Taylor for help in some of the experiments, and in preparing the work for publication.

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BLOOD VOLUME STUDIES

V. The Carbon Monoxide Method—Its Accuracy and Limitations

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Received for publication March 4, 1921

Many investigators consider the whole subject of blood volume to be in a state of hopeless confusion. Say our critics, how can we think otherwise when one set of observers report, the blood volume of dogs as 10 per cent body weight (dye methods), another as 8 per cent (CO method) and still others as 7 per cent (Welcker method). In this series of papers we submit experimental data which go far to explain these discrepancies and point the way to a blood volume method which is reasonably accurate (5 per cent error). In a word, our experimental data indicate that the blood plasma volume is accurately determined as 5 per cent (5 cc. per 100 gram body weight) by a number of dye methods. Furthermore our data show that the total body hemoglobin is accurately determined as approximately 4 per cent by the CO and Welcker methods. But in our opinion the true *total blood volume* is 5+ 4 = 9 per cent body weight, and not 10 per cent or 8 per cent respectively, calculating the hematocrit as 50 per cent. The *fundamental error, therefore, is the assumption that the ratio of cells to plasma is a constant* in all parts of the circulation. This we believe is contrary to fact. Experimental data and discussion of related facts are submitted below in their proper place (paper VII of this series).

It is not necessary to review the different methods and blood volume values as we can refer to a recent discussion by Salvesen (4). We may note in passing that the dilution blood volume methods of Cohnstein and Zuntz (1) which depend upon injection of isotonic sodium chloride solutions have no further usefulness. The recent experiments of Smith and Mendel (5) demonstrate the rapidity with which isotonic salt solutions leave the blood stream. Much of this solution may disappear

within 5 minutes. Some observations in this laboratory on dogs confirm this work of Smith and Mendel on rabbits.

It has been assumed by some workers that the wide divergence in blood volume values might be explained by differences in technical procedure. We thought it highly desirable that these various methods be used in this laboratory with a uniform technique, using the same healthy animals under controlled conditions. For this reason we have described in much detail the various technical procedures and subjected our methods to a variety of control tests.

The carbon monoxide method is very simple in principle. The CO is inhaled and a sample of blood is withdrawn for analysis to determine the amount of contained CO. This gives the amount of dilution effected by the circulating blood and so the total circulating hemoglobin or red cells. It is assumed that the CO is held only by the hemoglobin of the

TABLE 1

Known amounts of CO mixed with deoxidized blood and quantitatively recovered

EXPERIMENT	CO MIXED WITH BLOOD	READING AFTER PYROGALLIC ACID	READING AFTER COPPER SOLUTION	CO RECOVERED FROM BLOOD
	cc.	*cc.*	*cc.*	*cc.*
I	0.265	0.285	0.02	0.265
II	0.265	0.275	0.02	0.255
III	0.390	0.400	0.02	0.380
IV	0.310	0.330	0.02	0.310
V	0.330	0.345	0.02	0.325
Average.....	0.312			0.307

red blood cells, but there is good reason to believe that the hemoglobin of the red marrow and striated muscles (myohematin) participate in this dilution (see paper VII). The carbon monoxide method was first used by Gréhant and Quinquaud (2), later modified by many others and recently by Van Slyke and Salvesen (4), who report a simple method for the accurate analysis of small amounts of CO in blood. This last method with a few minor modifications has been used in our work.

Method of administering the carbon monoxide. The apparatus used for giving the CO gas corresponds in principle with that arrangement employed by Gréhant and Quinquaud (2), later modified by others, as reviewed in the introduction. The technique in detail is as follows: The gas, made by mixing and gently heating formic and sulphuric acids in tube *A*, figure 1, is first burned at *B* until it gives a bright blue flame. The three-way stopcock of the tube is then turned so that the gas bubbles through the bottles *C*, which contain concentrated NaOH

to remove any sulphur dioxide, carbon dioxide and water present. The carbon monoxide runs into the 100 cc. graduated burette *D*, previously filled with water and supplied with a leveling bulb, *D'*. This is connected by one arm of a three-way stopcock with a 50 cc. burette *E*, also previously filled with water and supplied with a leveling bulb, *E'*. The gas is run through these burettes and out through the arm of another three-way stopcock to *B* again, where about 200 cc. are burned, thus thoroughly rinsing out the connections. The two burettes are then filled with gas and all stopcocks closed except the one at *A* which is turned

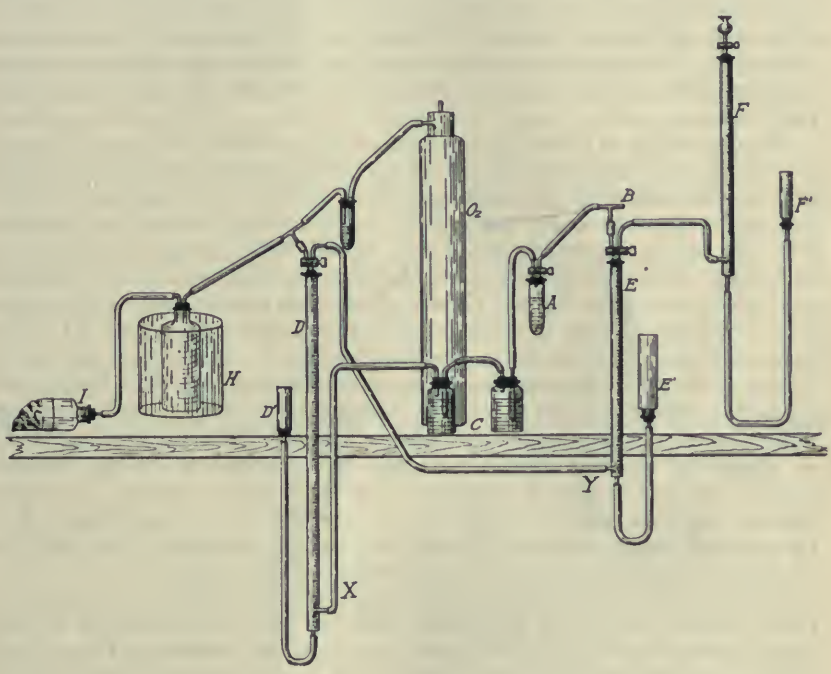

Fig. 1

so as to allow the CO still generating to escape to the outside. The connecting tubes are clamped at *X* and *Y*. The gas in *E* is measured at atmospheric pressure by means of the leveling bulb and 50 cc. are run up into the graduated cylinder *F*, which contains a solution of copper sulphate in concentrated hydrochloric acid (prepared by Sandmeyer's method). The solution on gentle shaking for a few seconds absorbs all the CO leaving behind the impurities, the volume of which is read directly at atmospheric pressure with the leveling bulb, *F'*. The gas is then run out of *F* by raising the leveling bulb and opening the stop-cock. The solution is thus ready for the next analysis.

The respiratory chamber, *H*, consists of a tank half filled with concentrated NaOH in which stands a bell-jar of about 2000 cc. capacity. The available gas

space is 1000 cc. This is connected with *D* through the other arm of the three-way stopcock and also with the oxygen tank, as shown in figure 1. It is also connected with the nosepiece, *I*. This consists of a glass bottle from which the bottom has been removed, of size varying according to the weight of the dog. A piece of automobile inner tubing of suitable diameter and length is slipped over the bottom of the bottle so as to form a cap for the dog's nose and protect the animal from the glass edge.

To prepare the respiratory chamber the air is sucked up to a mark, leaving about 500 cc. of air in the chamber and the tube to the hood is clamped. Oxygen is then allowed to enter in sufficient quantity to keep a slightly negative pressure within the respiratory chamber. This prevents any outflow of CO during the exhalation. The O_2 makes a smaller respiratory chamber possible and prevents any discomfort to the dog due to deficiency of oxygen. The apparatus is now ready for use.

The dog is tied on its back with the head at the edge of the table. The neck over the jugular vein is shaved and the nose is well greased with crude vaseline, as is also the inside of the nosepiece. This is slipped over the dog's nose to completely cover the mouth and nostrils and held firmly during the inhalation. The tube to the respiratory chamber is immediately unclamped. When all is adjusted, a measured quantity of CO (sufficient to saturate the blood corpuscles one-third if one assume 100 cc. of blood per kilogram body weight, of O_2 capacity 21 cc. per 100 cc. blood) is run in, and the tube rinsed with oxygen. The dog breathes into the respiratory chamber for 6 minutes. During this time as the volume of the gas in the chamber decreases, due to absorption of carbon dioxide, oxygen is supplied from the tank to keep the quantity of gas practically constant.

At the end of 6 minutes the nosepiece is removed. Four minutes later two 5 cc. samples of blood are drawn, by means of a hypodermic syringe, from the jugular vein and placed in tubes with powdered sodium oxalate to prevent clotting. A 10 cc. sample is then taken in a hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate (isotonic for blood). A sample of air from the respiratory chamber is taken and analyzed as described later.

The purpose of this method is to introduce into the animal's blood stream a known amount of CO. It is therefore essential that there should be no dead space and that the residue of CO left in the respiratory chamber and dog's lungs be accurately determined. The possible dead space is the tube from *D* to *H*. This is eliminated by the inhalations of the dog combined with the flooding of the tube with oxygen. The method employed in analyzing the respiratory chamber air will be discussed shortly. The entire procedure from making the gas to taking the samples is readily carried out and may be completed within 30 minutes.

Method of analyzing the samples of blood. The samples of blood are analyzed in the Van Slyke apparatus for blood gas analysis (6). The method employed is that described by Van Slyke and Salvesen (7). Our technique in detail is as follows: Three cubic centimeters of 0.4 per cent ammonia are introduced into the 50 cc. chamber of a carefully calibrated Van Slyke apparatus. This is followed respectively by 3 drops of caprylic alcohol and 3 drops of a 1 per cent aqueous solution of saponin. Following this 3 cc. more of the ammonia solution are added. The chamber is evacuated and shaken a few times to extract

the air. This is expelled and the process repeated to make certain that all the air is removed and that there is no leak around the stopcocks. On opening the lower stopcock, the solution should rise with a click to the top. Two cubic centimeters of this solution are run up into the cup. A 2 cc. Ostwald pipette, calibrated for complete delivery, is used to introduce the sample of blood into the cup under the ammonia solution. The blood and supernatant liquid are run into the chamber and the cup rinsed with a few drops of ammonia solution. One minute is allowed for laking. One cubic centimeter of a saturated potassium ferricyanide solution is then added, the stopcock sealed with mercury, and the apparatus evacuated until only about 2 cc. of mercury remain in the 50 cc. chamber. The machine is shaken for 15 minutes by a motor which throws the solution gently with a rotary motion against the sides of the chamber, but does not shake so violently as to emulsify the mercury. We found inconsistency in the readings of CO attendant on the variations in the length and severity of shaking, and established this technique after carefully controlled experiments.[1] After taking the apparatus off the shaking machine, the lower stopcock is opened and 1.5 cc. of pyrogallic acid solution (12 grams in 72 cc. of a 33 per cent NaOH solution) are put in the cup and run in a little at a time to absorb the oxygen. When the reading is constant the solution is trapped as completely as possible in the bulb below the lower stopcock, the graduated tube is rinsed clean with water, which also permits the reading of the bottom of the meniscus, previously impossible on account of the turbidity of the solution. That no leak has occurred during the procedure is verified by running in a little of the copper solution. This absorbs all but about 0.025 cc. of the gas. The analysis is duplicated. Previously we have been satisfied to obtain duplicate readings which vary by as much as 0.01 cc. With further experience they now rarely vary more than 0.005.

Discussion of the method of analyzing the blood. The accuracy of this method depends on the technique of the person working with the instrument. To check this we did a set of controls (table 1). About 2 cc. of blood are run into the 50 cc. chamber which is then evacuated and the blood shaken. By this procedure the oxygen in combination with the hemoglobin is removed. This may be run out of the machine at intervals. Within 20 minutes the blood is almost completely deoxidized. The blood is then trapped in the lower bulb and the 50 cc. chamber washed out. The connections are rinsed with CO gas and about 0.2 or 0.3 cc. of CO is introduced and washed in with a few drops of water to empty the bore of the stopcock and give a concave meniscus.

[1] Since sending this paper to press, an article by Theo. K. Kruse in the American Journal of Physiology, Volume lv, number 2, page 289, has come to our notice. In conformity with his results, we find that the long shaking is unnecessary, if, after adding the potassium ferricyanide, and sealing, the solutions are allowed to stand in contact for ten minutes. The apparatus is then evacuated and shaken three minutes longer.

The blood is then released from the lower bulb into the upper chamber and mixed with the gas. With gentle shaking the CO is soon completely absorbed. Six cubic centimeters of the ammonia saponin and caprylic alcohol solution, freed of its gas content in a second Van Slyke instrument, are then poured into the cup and let in to the chamber. The routine analysis is made. As the figures in table 1 indicate, the amount of CO recovered corresponds (within the limit of error of our technique) to that introduced into the machine.

TABLE 2

Blood analyses performed immediately and after 24 hours both with and without oil

EXPERIMENT	SAMPLE DRAWN WITHOUT OIL		SAMPLE DRAWN UNDER OIL	
	At once	After 24 hours	At once	After 24 hours
	cc.	cc.	cc.	cc.
I	0.202	0.200	0.200	0.200
II	0.158	0.160	0.158	0.157
III	0.185	0.180	0.182	0.181
IV	0.238		0.240	
V	0.194	0.190	0.192	
VI	0.180	0.175		
VII	0.190	0.185		
VIII	0.140	0.140		
IX	0.260	0.260		
X	0.123	0.125		

Accuracy in the method of analyzing the blood is, however, useless if gas is lost either in handling the samples of blood or by escape from the blood stream through the lungs or into the tissues. It has been formerly customary to draw the blood under oil and take care that it should not be exposed to the air at any time because of diffusion of the gas. Table 2 shows the result of a series of experiments in which samples drawn under oil are compared with those drawn without oil, both when the samples are analyzed immediately and after an interval of 24 hours. It will be seen that the loss by diffusion from either sample even over a period of 24 hours is not appreciable.

Table 3 shows the result of a series of experiments to determine the interval of time, after removing the nosepiece, which may elapse before the analysis of the blood indicates an appreciable decrease in content of CO. Samples were drawn either while the hood was still on or within 1 or 2 minutes of removing it. Samples were then taken after 4, 7 and 10 minutes. Only the last shows a drop in the reading. We

have, therefore, chosen 4 minutes as a routine length of time to allow complete, even distribution of the gas. Haldane and Smith (3) discuss the possible loss of gas by physical solution in the body fluids. The tension in the blood is so low (0.2 per cent, that supposing all body fluids to be saturated at this same tension the quantity of CO so bound is less than 1 cc.

TABLE 3

Readings of samples of blood drawn at varying intervals after inhalation of CO

DOG NUMBER	WEIGHT	CARBON MONOXIDE LIBERATED BY 2 CC. OF BLOOD			
		1 to 2 minutes	4 minutes	8 to 9 minutes	10 minutes
	kgm.	*cc.*	*cc.*	*cc.*	*cc.*
19–128	27.2	0.200	0.200	0.200	0.190
19–128	27.2	0.175	0.180	0.185	0.175
18–4	18.2	0.160	0.160	0.160	0.155
18–4	18.2	0.194	0.190	0.182	0.178
21–11	29.5	0.195	0.195	0.195	0.195
19–102	18.2	0.205	0.202		0.193
21–6	10.4	0.210	0.210		0.200
Average		0.191	0.191		0.184

Method of calculating the blood volume. To calculate the blood volume, first reduce the volume of gas inhaled to standard conditions by multiplying by the factor $(0.999–0.0046\ t) \times \frac{\text{barometer}}{760}$ where t is the temperature in degrees centigrade. From this must be subtracted the CO remaining in the respiratory chamber, as determined below.

The reading of the gas obtained from the blood analysis may be converted directly into cubic centimeters of CO per 100 cc. (volume per cent) of blood by using table 1 of Van Slyke's paper on oxygen (8). There is a small amount of nitrogen which is derived from the physically dissolved air in the blood and is read along with the CO after the pyrogallic acid has absorbed the oxygen. So to correct for this 1.2 must be subtracted from the volume per cent derived above as determined by Van Slyke (7). This may either be corrected for as stated, or eliminated as mentioned previously, by absorbing the CO with the copper solution and thus reading directly the true CO volume. Having now the total gas absorbed and the number of cubic centimeters of this recovered in 100 cc. of blood, the former divided by the latter gives the total blood volume. The hematocrit gives the proportion of cells to plasma, while from the weight of the dog the cubic centimeters of blood per kilogram body weight may be calculated. The reading of CO obtained in the Van Slyke apparatus is directly proportional to the cell per cent hematocrit and the blood volume is inversely proportional to the reading. It of course is obvious, therefore, that a hematocrit representative of the entire blood volume is not necessary, but it is necessary that the

sample for the hematocrit and the sample to be analyzed should be taken at the same time so that the per cent of cells in each is the same. If this is the case the true measure of the body hemoglobin will be obtained, though the plasma volume and total volume will be inaccurate according as the hematocrit fails to be representative of the per cent cells in the whole blood.

Analysis of the gas remaining in the respiratory chamber. The principle of the method of analysis employed is to absorb the oxygen from a 100 cc. sample of the gas by means of pyrogallic acid solution. The remaining gas is then mixed with 2 cc. of blood, deoxidized as described before. This oxygen-free blood absorbs the CO which is then determined by the routine analysis of the blood sample. The technic in detail is as follows: A 100 cc. sample of the gas is drawn into a graduated tube of capacity slightly over 105 cc., one end of which is provided with a three-way stopcock, while the other end is connected with a leveling bulb. Mercury is used to adjust the pressure. The tube resembles a urea tube and is graduated only in 5 cc. intervals. Five cubic centimeters of pyrogallic acid are added through one arm of the three-way stopcock and the whole apparatus is shaken at short intervals to facilitate absorption of the oxygen. Meanwhile a little over 2 cc. of blood is deoxidized in the Van Slyke gas apparatus. In about one-half hour the reading in the tube has become constant. The two are connected with rubber tubing and the connections are filled with mercury. As much gas as the analyzer will hold, at atmospheric pressure, is run over and mixed with the blood. Gentle rotating to spread the blood over the walls of the chamber, with remixing of the gas in the tube and Van Slyke apparatus at 3 minute intervals, insures practically complete absorption of the CO within 45 minutes. The blood is then analyzed by the routine technic—the gas-free ammonia solution being introduced as above. An average of those analyses given in table 4, in which the per cent of residual O_2 is approximately 30 per cent, gives, with a calculated total chamber and lung volume of 1500 to 2000 cc. (depending on the size of the dog) approximately 2.00 cc. of unabsorbed CO. The total volume of lungs and respiratory chamber is estimated rather than measured, as by Gréhant and Quinquaud, as the correction would be at most less than 1 cc. of gas, which is a degree of precision the accuracy of the method does not permit.

The limited number of experiments so far performed suggests that the amount of CO remaining in the respiratory chamber is a factor of the per cent of O_2 found in the jar. Thus table 4 shows that the residual CO is doubled or tripled when the per cent of O_2 is between 40 and 50. The amount of O_2 introduced into the respiratory chamber is now regulated so as to constitute approximately 30 per cent of the total volume. Under these conditions the residual CO amounts to about 2 cc., which figure we now use in calculating the volume, as mentioned above.

This method is not the one first employed in the laboratory, nor is it identical to those described in the literature. It has finally been adopted because it is reasonably easy to carry out and may readily be controlled. Two series of controls were run. First, 100 cc. of air

from the room or out of doors were analyzed in the same way. It was found that on an average 0.08 cc. of a gas, which was not absorbed by pyrogallic acid but was absorbed by the copper solution, was obtained. The gas was shown finally to be derived from the pyrogallic acid then in use. Subsequent experiments with other samples of pyrogallic acid gave at most 0.01 cc. of such gas. The second series of controls consisted in measuring in the analyzer a small quantity of CO, 0.2 to 0.3 cc., and

TABLE 4

Analyses of 100 cc. samples of gas from respiratory chamber taken immediately after blood volume determination

EXPERIMENT	DOG NUMBER	O_2 IN RESIDUAL GAS	CO IN 100 CC. GAS	TOTAL RESIDUAL CO
		per cent	*cc.*	*cc.*
I	21–30	20	0.060	1.00
II	21–10	45	0.340	5.61
III	19-102	50	0.317	5.68
IV	21–5	44	0.310	4.65
V	19–128	30	0.110	2.20
VI	18–4	30	0.110	2.03
VII	21-6	30	0.110	1.80

TABLE 5

A known amount of CO mixed with 100 cc. of air. Quantitative recovery of CO by method described

EXPERIMENT	CO MIXED WITH 100 CC. OF AIR	CO RECOVERED IN BLOOD MIXTURE
	cc.	*cc.*
I	0.15	0.140
II	0.21	0.205
III	0.16	0.185
IV	0.31	0.314
Average.....	0.208	0.211

of running this into the tube with 100 cc. of air. This gas was then analyzed by the same procedure as in the case of the respiratory chamber air. The tabulated results in table 5 show that the CO is completely recovered by this procedure.

Hemorrhage control experiments. The following hemorrhage experiments were carried out as a control of the CO blood volume method. If the CO method is accurate it should be possible to determine the hemoglobin volume before and after unit bleedings of known amounts and

show that the measured and calculated hemoglobin values compare with reasonable accuracy. In all these experiments the control determination was made the day previous to the hemorrhage. The second blood volume determination was done immediately following the hemorrhage. The determination of blood volume was made from 4 minutes to 1.5 hours following the completion of the hemorrhage, depending on the condition of the animal after the bleeding. In each instance the blood was drawn by means of a needle in the jugular vein into 1.6 per cent sodium oxalate, measured, and the hematocrit values determined from a 10 cc. sample. Anesthesia was not used in these experiments which cause no discomfort to the dogs except the puncture of the skin by the hypodermic needle.

TABLE 6

Hemorrhage experiment

Dog 19–98. Young, active male. Weight, 55 pounds

DATE 1920	TIME	RED CELLS HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 23	11:30 a.m.	55.9	**974**	768				
February 24	4:30 p.m.	49.4			624	308	316	
February 24	5:30 p.m.	42.2	**678**	928				**666**
February 25	4:30 p.m.	32.3			615	199	416	
February 25	5:15 p.m.	30.8	**535**	1202				**467**
February 26	2:30 p.m.	27.5	**461**	1216				
February 27	1:15 p.m.	25.7	**452**	1307				
February 28	12:00 m.	27.1	**524**	1408				
March 5	2:00 p.m.	34.0	**833**	1616				
March 9	3:30 p.m.	39.0	**856**	1338				

20 cc. whole blood drawn for each determination.

Table 6 presents a typical experiment on a normal dog which was bled large amounts on two successive days. About one-third the hemoglobin volume was removed in each bleeding. The last column gives the expected or calculated hemoglobin or red cell volume. It is noted that the calculated and measured red cell volumes following the first bleeding are practically identical, 666 cc. and 678 cc. respectively. Following the second large hemorrhage, we note the measured cell volume is 60 cc. greater than the calculated cell volume. On the following day the hemoglobin volume has fallen to the expected level and one is inclined to explain the 2nd day's figures as due in part to an error in technique. However, there are physiological variations in these figures

which cannot be discussed at this time but may explain some of these fluctuations. Following the low level of the two days after the hemorrhages, we note a steady gain in hemoglobin volume. It may be noted that the amount of blood removed for each analysis (20 cc.) is allowed for in the bleeding periods and recorded figures. The calculated volume in each instance is obtained by subtracting from the normal cell volume the actual amount of red cells removed during the bleeding and experimental analysis.

TABLE 7

Hemorrhage experiment

Dog 20–37. Young, active, female. Weight, 22 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 16	3:30 p.m.	50.8	**433**	420				
February 17	3:30 p.m.	50.3			250	126	124	
February 17	5:30 p.m.	39.9	**322**	483				**307**
February 18	4:00 p.m.	30.6	**310**	704				
February 19	4:15 p.m.	29.8	**310**	731				
February 20	2:30 p.m.	30.6	**302**	684				
March 12	7:50 a.m.	55.0	**418**	342				

20 cc. whole blood drawn for each determination.

Table 7 shows a single hemorrhage experiment. The calculated and measured red cell volume correspond very closely, 307 cc. and 322 cc. respectively. Following this bleeding there is not a prompt response nor any appreciable amount of hemoglobin constructed during the 3 days following the large hemorrhage. The daily bleeding of 20 cc. may have disturbed the normal body reaction tending to reproduce hemoglobin and red cells and we must assume that hemoglobin construction was practically stationary. In general we may say that following these large bleedings there was little evidence of very active reconstruction of red cells and hemoglobin during the first period of 2 to 5 days. It is more than probable that the daily bleedings of 20 cc. whole blood plus the CO inhalation actually inhibited the normal reaction which we believe is prompt and productive under normal conditions.

Table 8 shows an experiment very similar to that recorded in table 6. After the first bleeding the calculated and measured red cell volume correspond closely, the measured volume exceeding the calculated by 20 cc. After the second large bleeding we note again the excess of

the measured volume over the calculated hemoglobin volume as 36 cc. This might be explained as a temporary emergency reserve reaction due to mobilization of hemoglobin normally retained in sluggish or isolated sinusoids of the spleen and red marrow. The reaction is similar in all these three experiments. Also there is a fall to the expected value on the days following the hemorrhages. The several days following the two bleedings in the experiment show periodic fluctuations in red cell volume but no significant rise in hemoglobin values. We hope to come back to this point again in subsequent publications.

TABLE 8

Hemorrhage experiment

Dog 19–66. Young, active male. Weight, 29 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 2	4:30 p.m.	59.6	**573**	388				
February 3	3:00 p.m.	58.1			330	192	138	
February 3	3:14 p.m.	56.7	**403**	307				**383**
February 4	4:20 p.m.	35.5			330	117	213	
February 4	5:04 p.m.	34.9	**300**	560				**264**
February 5	3:30 p.m.	25.5	**261**	761				
February 6	3:30 p.m.	26.2	**324**	911				
February 9	12:15 p.m.	30.5	**300**	683				
February 12	3:00 p.m.	32.8	**318**	650				
March 11	2:30 p.m.	52.4	**528**	479				

20 cc. whole blood drawn for each determination.

Table 9 shows an experiment with two large bleedings on consecutive days. This experiment gives the measured values as *lower* than the calculated red cell volumes. We may explain this apparent exception to our other experiments as due to a slight error in the base line or normal hemoglobin values. The difference, however, is not great and the final hemoglobin volume of 179 cc. is very close to the calculated value of 184 cc. This dog, unlike the others, shows a distinct gain in hemoglobin volume in the few days after the hemorrhages.

The experiments in table 10 show how closely the cell volumes agree when the determinations are made on the same animal at different periods. The animals were all young healthy adults. Puppies show certain variations which are being studied at present and will be reported at some future time. In two instances here two determinations

TABLE 9

Hemorrhage experiment

Dog 20-34. Young, active male. Weight, 23 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
April 13	3:30 p.m.	58.0	**434**	315				
April 14	3:00 p.m.	55.0			260	143	117	
April 14	3:10 p.m.	49.2	**265**	273				**291**
April 15	3:20 p.m.	41.0			260	107	153	
April 15	3:40 p.m.	35.9	**179**	319				**184**
April 16	2:00 p.m.	29.1	**233**	568				
April 20	2:30 p.m.	36.0	**292**	519				

20 cc. whole blood drawn for each determination.

TABLE 10

Blood volume determinations on normal dogs. CO method—red cell volume

DOG NUMBER	DATE	WEIGHT	HEMATOCRIT	RED CELLS	PLASMA	BLOOD PER 100 GRAMS	CELLS PER 100 GRAMS
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*
19–66	December 29, 11:15 a.m.	13.2	63.4	**602**	347	7.21	**4.57**
19–66	December 29, 4:15 p.m.	13.2	60.3	**604**	355	7.28	**4.39**
19–66	February 2	13.2	59.6	**573**	388	7.30	**4.36**
19–137	December 10	12.9	50.6	**540**	528	8.25	**4.17**
19–137	December 11, 3:30 p.m.	12.9	53.4	**563**	491	8.14	**4.37**
19–137	December 11, 4:45 p.m.	12.9	48.9	**567**	593	8.97	**4.39**
19–137	December 16	12.9	50.8	**536**	520	8.16	**4.15**
20–37	February 16	10.0	50.8	**433**	420	8.54	**4.34**
20–37	March 12	10.4	55.0	**418**	342	7.28	**3.68**
21–5	August 6	6.6	46.3	**249**	289	8.16	**4.38**
21–5	August 9	7.0	43.7	**239**	308	7.77	**3.89**
21–5	August 17	7.2	43.3	**244**	320	7.89	**3.94**
21–5	August 18	6.6	43.0	**243**	323	8.60	**4.26**
21–5	September 28	6.9	52.3	**253**	231	6.99	**4.09**
21–5	October 18	7.0	52.4	**253**	230	6.86	**4.02**
21–5	October 21	7.2	50.3	**246**	254	6.84	**3.86**
Average				**454**	389	7.78	**4.20**

were made on the same animal during the same day. In both of these cases there was a slight residue of CO gas remaining in the blood when the second determination was carried out. This was corrected by making a CO analysis with a sample of blood drawn immediately before

determining the second blood volume. The variation in cubic centimeters of blood per 100 grams of animal's body weight is due to the variation in the plasma volume determination. The cell volume varies within experimental limits. In other words, here again is evidence of a reasonably correct cell volume determination by the CO inhalation method as outlined above.

DISCUSSION

We wish to stress the fact that the carbon monoxide method gives a true measure of the pigment substances of the body which hold CO so tenaciously. These pigment substances include the hemoglobin of the circulating blood and probably that in the stagnant sinusoids of the spleen and red marrow. It is very probable that the muscle hemoglobin (myohematin) is also included in these pigment substances which dilute rapidly the inhaled carbon monoxide. We believe then that this CO method gives an accurate measure of this *body hemoglobin* but we fall into decided error when we *calculate* the total blood volume from the red cell hematocrit. The correctness of this calculation depends on the assumption that the blood hematocrit obtained from the jugular vein is representative of all parts of the body circulation, including arterioles and capillaries. We believe these figures obtained by *calculation* are *incorrect for the plasma volume* which is actually much larger than the hematocrit calculation would indicate.

In this paper we use the term *hemoglobin volume* to indicate the pigment substances which are concerned in the dilution of the inhaled carbon monoxide. It is evident that we do not believe that this figure represents only the actively circulating hemoglobin in the red cells but this circulating hemoglobin, of course, makes up the greatest part of the figure (90 to 95 per cent).

The hemorrhage experiments in which unit amounts of blood are removed, the body hemoglobin being measured before and after the single or double bleedings, give strong evidence of the accuracy of the method employed. The calculated and actual measured values correspond very closely. Moreover, repeated determinations on normal dogs under uniform conditions give figures which are quite uniform. All this indicates that the method of estimation is accurate provided we understand the fact that we are measuring only the *body hemoglobin* and *not the plasma volume.*

SUMMARY

The carbon monoxide method gives a reasonably accurate measure of the *body hemoglobin* and *hemoglobin volume*. We use these terms to indicate the total body pigment substances which fix the inhaled CO and bring about its dilution in the circulating blood. It is obvious that the true red cell hemoglobin volume is slightly less than the total hemoglobin volume.

Repeated hemoglobin volume estimations on normal dogs give figures which are reasonably constant, allowing for certain expected physiological variations.

Hemoglobin volume estimations before and after unit hemorrhages give figures for the actual *measured* and indirectly *calculated* amounts which are in close accord

In our opinion this method alone gives no accurate information concerning *plasma volume*.

Various controls of the several steps of this method, as used in our hands, are submitted. These controls establish this method as accurate at least within 5 per cent error. Further refinements and the use of packed red cells, especially in anemia, increase the accuracy of the method.

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BLOOD VOLUME STUDIES

VI. Plasma Volume as Determined by Hemoglobin Injection

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Received for publication March 4, 1921

The hemoglobin method is exactly like the vital dye method and consists in the introduction intravenously of a known amount of a fresh hemoglobin solution. After a 4 minute period to permit of uniform distribution of the hemoglobin in the circulating blood a sample of blood is drawn into isotonic oxalate. From this plasma sample is determined colorimetrically the degree of dilution of the known hemoglobin introduced and the blood plasma volume is directly measured.

We wish to repeat that this method and many other similar methods give reasonably accurate figures for the blood *plasma volume* but we may not assume that the calculated red cell volume as determined by the hematocrit is also accurate. This point will be elaborated in the following paper.

All our experimental data point to a relatively *constant blood plasma volume* in adult dogs under uniform conditions. Estimated by the hemoglobin or dye methods the normal blood plasma volume of an adult healthy active dog is very close to 4.9 to 5.0 cc. per 100 gram body weight. There are normal variations due to age, physical condition and physiological activities which we hope to review in the near future. At this point we may refer to observations made in this laboratory by means of the dye method (3), (2), (7).

One need not be surprised at certain fluctuations in the plasma volume as determined by the hemoglobin or dye methods. On the other hand there is more reason for surprise at the constant figures obtained under certain conditions. When we contrast circulatory conditions in resting dogs with those of great muscular activity or digestive activity we may well wonder at the mechanism which shuts off one mass of body capillaries as another is opened to great activity. One can im-

agine that the greater part of spleen and red marrow sinusoids may at times be very incompletely washed with circulating plasma or again actively a part of the circulation. There is reason to believe that this constant circulating blood plasma is one factor in itself which is concerned with the partial isolation of one capillary field as another is opened for activity (digestion, for example). The *blood proteins* may be concerned in this adjustment complex and it has been pointed out in publications (4), (5) from this laboratory that the body will not tolerate great reductions in the concentration of the blood plasma proteins; further that the body can form these serum proteins only with difficulty and slowly. If the blood plasma proteins regulate circulating plasma fluctuations we may say that the body tissues which produce or replace the plasma proteins may take part in the regulation of circulating plasma volumes. We hope to supply experimental data which will help to solve these problems.

Some critics say that the dye method may be inaccurate because of the fact that a chemical substance foreign to the body is introduced into the blood stream. The very presence of this dye may modify circulatory conditions although admittedly there is no evidence of any cardio-vascular disturbance. To answer this objection hemoglobin is an ideal substance as it is not foreign to the blood stream and may be given intravenously in considerable amounts to dogs and man without the slightest clinical disturbance. Hemoglobin was first used in this way by Barratt and Yorke (1). The accuracy of the hemoglobin method is largely dependent on our knowledge that no hemolysis of red cells is caused at any step in the procedure. We have controlled this point in our experiments as given below. It is significant that the dye method and the hemoglobin method give identical figures for blood plasma volume.

Method. The general technical procedure in these experiments is identical with that previously described in work on blood plasma volume with vital dyes (3). The hemoglobin solution used in our work is prepared in the following manner: About 12 cc. of blood from the jugular vein of a dog are emptied into each of two or three 15 cc. centrifuge tubes containing about 2 cc. of 1.6 per cent oxalate solution. These tubes are then centrifuged for 30 minutes at 2500 revolutions per minute. After removing the plasma, 6 cc. of the packed red cells are mixed and shaken for 5 minutes with 30 cc. of distilled water. This makes a dilution of 1 cc. of packed cells in 6 cc. of fluid, which is a concentration at which the red cells are completely laked. This solution is clear and contains a negligible amount of stroma which will settle out on centrifugalization or on standing over night. In most of our experiments this hemoglobin solution is not centrifugalized but

allowed to stand over night in the ice chest. Twenty-five cubic centimeters of the hemoglobin solution is the amount found to be satisfactory for determining the plasma volume of an average dog. More or less may be used, depending upon the size of the dog, and the individual taste in color readings.

In testing this method only normal adult dogs were used. A clean hypodermic needle is inserted into the jugular vein, and about 12 cc. of blood drawn in a clean syringe previously rinsed in 0.9 per cent saline. This sample of blood is then emptied carefully without bubble formation to prevent hemolysis, into a 15 cc. graduated hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate solution. The syringe is then rinsed in 0.9 saline and another sample of blood drawn and emptied into another oxalated hematocrit tube similar to the first. The blood and oxalate are mixed by inverting the tubes twice, which are then stoppered. Twenty-five cubic centimeters of hemoglobin solution, which has previously been measured into a porcelain dish using a calibrated pipette, are drawn up into a large syringe along with 5 to 10 cc. of 0.9 per cent saline used to wash out the porcelain dish. This solution is now injected slowly into the jugular vein and the hemoglobin rinsed out of the large syringe by withdrawing and reinjecting a little blood. The needle which has been in the vein during this process is allowed to remain, while about 10 cc. of saline are slowly injected to prevent clot formation in the needle. Exactly 4 minutes after injection of the hemoglobin solution a sample of blood is drawn in a clean syringe previously rinsed with 0.9 per cent saline, and emptied into a 15 cc. hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate. Immediately the syringe is rinsed in 0.9 per cent saline and a second sample is collected in a similar manner. Both the tubes are then inverted twice to insure thorough mixing, and stoppered. All four tubes are now centrifugalized at 2500 revolutions a minute for 30 minutes. The total contents and the number of cubic centimeters of cells are then noted. The red cell hematocrit normal values are computed from the control tubes.

The hemoglobin colored plasma is read in a Hellige's colorimeter against a standard prepared as follows:

1. One cubic centimeter of the hemoglobin solution from which 25 cc. were taken above for injection, is pipetted into a clean test tube to which is then added exactly 9 cc. of distilled water, thus making a 10 per cent hemoglobin solution.

2. Five cubic centimeters of the above 10 per cent solution are then diluted with 10 cc. of clear plasma obtained from the first two samples of blood drawn from the dog. Thus 0.5 cc. of the hemoglobin solution is diluted in 15 cc. of the standard.

Against this standard the colored unknown plasma is read and expressed in per cent. In the formula below let this reading be referred to as R. The number of cubic centimeters of hemoglobin solution injected into the dog is referred to as D, and the correction for the dilution by the oxalate present in the tinted plasma as C. Thus C expresses the ratio between the actual concentration of color in the plasma when diluted with 2 cc. of oxalate solution to the value when not so diluted.

The standard for color comparison contains 1 cc. of hemoglobin solution in 30 cc. of fluid.

Therefore D cc. of the hemoglobin solution (amount injected) will impart the same color intensity to 30 D cc. of fluid.

If, however, the color intensity is $\frac{R}{100\ C}$ (i.e., the colorimetric reading in per cent corrected for the dilution of the plasma by the oxalate solution).

Then, the number of cubic centimeters of fluids that have been tinted by D cc. of hemoglobin solution $= \frac{(30\ D)\ (100\ C)}{R}$ or, the *plasma volume* in cubic centimeters $= \frac{3000\ D\ C}{R}$.

Unless otherwise stated all experiments were done upon healthy dogs. A mixed diet of table scraps, including bones, meat, bread, potatoes, rice, etc., was given daily. Water was in the cages at all times.

Method control. If any hemolysis of red cells is produced by any of the steps of this method then the method has little if any value. Dogs' corpuscles are notoriously fragile and all workers in this field should control the method in their hands under local laboratory conditions. For this reason all specimens are drawn in duplicate and all readings of these duplicate specimens must check. Blood should be emptied down the sides of centrifuge tubes as air bubbles often cause slight hemolysis. For the same reason the last 1 to 2 cc. of blood in the syringe which may contain air bubbles should be discarded.

Our solution of hemoglobin is not made isotonic. Does this hypotonic solution by itself cause any hemolysis in the circulation? We controlled this step and others in the method by the injection of distilled water into the vein instead of the hemoglobin solution. In all other respects the blood samples, etc., were handled in the usual fashion described above. Two samples of blood were drawn into isotonic sodium oxalate after a period of 4 minutes following the injection of 25 cc. of distilled water. Six dogs injected in this way with 25 cc. of distilled water showed water-clear blood plasma obtained in the usual way. There was no hemolysis.

Other experiments in which larger amounts of distilled water were injected intravenously show that a very faint trace of hemolysis is observed only when the distilled water injected amounts to approximately 10 per cent of the total plasma volume. It is obvious therefore that we are within safe limits when hypotonic solutions in amounts less than 5 per cent of plasma volume are used for intravenous injection.

Experimental observations. One of the first things to be established for the hemoglobin method, as for the dye methods, is the curve of disappearance of the introduced hemoglobin. It will be noted that the

hemoglobin leaves the circulation less promptly than the vital dyes but unlike the vital dyes is completely removed from the plasma within 18 hours. The body has other methods of dealing with hemoglobin in the circulating plasma as compared with vital dyes which persist in the blood stream for many days, depending in part upon the amount injected (2). For certain purposes this complete removal of the hemoglobin from the blood plasma within 18 hours is of especial importance and makes the hemoglobin method of distinct value for certain experimental work.

TABLE 11

Rate of elimination of hemoglobin solution from circulating blood plasma

DATE 1920	DOG NUMBER	WEIGHT	2 MINUTES	4 MINUTES	10 MINUTES	30 MINUTES	2 HOURS
		kgm.					
February 2	20–80	15.91	100	101	96	92	74
February 17	20–80	18.17	100	99	96	86	75
February 18	20–80	17.95	100	100	94	86	60
February 19	18–4	20.21	100	102	97	96	78
February 20	18–4	20.11	100	100	99	91	
February 25	18–4	20.45	100	98	94	92	78
Average				100	96	91	73

Table 11 gives the figures for the decrease in concentration of the injected hemoglobin in the circulating plasma. In all experiments 25 cc. of the standard hemoglobin solution were injected into the jugular vein. The samples removed at the end of 2 minutes are figured as 100 per cent—all samples in duplicate. It is clear that the hemoglobin remains in the blood stream with a minimal removal during the first 10 minutes. We think it is safe to assume that a 4-minute sample will give values which should be within 2 to 3 per cent of the true dilution if there was absolutely no escape of hemoglobin in the circulation. We consider that this figure is within the accuracy of the colorimetric determination.

After 1 to 2 hours the plasma samples take on a slightly brownish tint which makes accurate color comparisons difficult. This is undoubtedly due to the change of circulating hemoglobin into bile pigment substances (6), which is known to take place rapidly in dogs. This reaction is a recognized body function and makes for a complete and rapid removal of the injected hemoglobin, so that the plasma figures after 18 hours are zero. It is possible by the hemoglobin method to repeat

such blood volume observations at relatively short periods without the troublesome complication of a residual plasma dye.

Table 12 includes many observations on young adult, active, healthy dogs. Several observations on the same animal under different dates are recorded. There are considerable variations in body weight and plasma volume. The dogs were all on a diet of mixed table scraps but no effort was made to insure a constant food intake and body weight in these experiments. The average for this group of dogs is 51.8 cc.

TABLE 12

Blood plasma determination on normal dogs

DATE 1920	DOG NUMBER	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
January 22	20–80	17.85	48.6	**921**	871	1792	100.4	**51.6**
January 30	20–80	16.81	47.4	**918**	855	1773	105.5	**54.6**
February 17	20–80	18.17	45.8	**1057**	895	1952	107.4	**58.1**
April 7	20–73	16.36	55.8	**741**	934	1675	102.4	**45.3**
February 9	18–4	19.88	56.7	**928**	1218	2146	107.9	**46.7**
February 19	18–4	20.21	55.4	**993**	1161	2154	106.5	**49.1**
February 20	18–4	20.11	49.1	**1089**	987	2076	103.2	**54.1**
February 25	18–4	20.45	47.0	**1113**	1001	2114	103.3	**54.4**
March 23	18–4	19.08	55.4	**868**	1076	1944	101.9	**45.5**
April 19	18–4	18.40	48.6	**1082**	1020	2102	114.2	**58.8**
August 27	19–102	16.82	50.7	**876**	900	1776	105.6	**52.1**
Average		18.55	50.9	**962**	992	1954	105.3	**51.8**

plasma per kilo body weight. Calculated blood volume on the basis of a 50 per cent hematocrit introduces the error already referred to. These figures correspond to those recorded in this laboratory with the brilliant vital red method.

Table 13 is very much like the preceding table but these dogs are all much heavier. It is a constant observation in this laboratory that these large, heavy, quiet dogs give lower plasma volumes than do the active, muscular dogs of smaller size. A part of this plasma volume decrease undoubtedly is due to the increase in fat which is less rich in capillaries than other body tissues like muscle. The plasma volume, therefore, may be expected to decrease per kilo as a dog puts on fat and becomes less active. Our highest blood volume figures per kilo are recorded in lean, very active, young adult dogs. The blood plasma figures are apt to be more constant in the larger dogs—see dog 19-128, table

13. Compare the average plasma volume per kilo = 51.8 cc. for the active dogs, and the plasma volume per kilo = 41.0 cc. for the large, fat dogs (table 13).

TABLE 13

Blood plasma determination on normal large dogs

DATE 1920	DOG NUMBER	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
March 17	19–128	28.17	55.5	**1064**	1339	2403	85.4	**37.7**
March 25	19–128	27.85	56.3	**1099**	1416	2515	90.3	**39.4**
April 7	19–128	28.52	55.5	**1080**	1349	2429	85.1	**37.8**
April 29	19–128	27.40	57.2	**1136**	1518	2654	96.8	**41.4**
June 7	19–128	26.70	60.5	**1143**	1749	2892	108.3	**42.8**
July 13	19–128	27.70	62.5	**1122**	1872	2994	108.1	**40.5**
July 20	19–128	27.50	61.6	**1137**	1824	2961	107.7	**41.3**
April 14	17–160	26.92	60.5	**1143**	1752	2895	107.4	**42.4**
June 24	17–160	24.18	50.4	**1159**	1179	2338	96.7	**47.8**
June 21	18–60	24.10	52.9	**976**	1095	2071	86.0	**40.5**
August 20	17–154	23.20	57.8	**995**	1345	2340	100.8	**42.8**
August 27	21–11	29.21	59.1	**1149**	1661	2810	96.2	**39.3**
Average		26.78	57.4	1099	1509	2608	97.4	**41.0**

TABLE 14

Daily blood plasma determination

DATE 1920	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
	kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Experiment A. Dog 18–4. Wire-haired mongrel. Lean and active female							
June 14	19.6	50.2	**1057**	1068	2125	108.7	**53.9**
June 15	19.6	48.0	**1100**	1053	2153	110.1	**56.1**
June 16	19.6	48.0	**1084**	1003	2087	106.8	**55.3**
June 17	19.6	45.8	**1220**	1032	2252	115.1	**62.2**
Experiment B. Dog 17–160. Large mongrel bull. Fat and inactive male							
June 14	25.3	53.6	**1120**	1294	2414	95.9	**44.2**
June 15	25.2	48.9	**1138**	1100	2238	88.8	**45.2**
June 16	25.1	48.5	**1203**	1134	2337	93.2	**47.9**
June 17	24.6	52.1	**1163**	1265	2428	98.9	**47.2**

Table 14 gives observations on two normal dogs with daily blood plasma determinations for 4 days. The dogs were fed the usual mixed diet in the afternoon after the blood volume determinations had been

completed. They had access to water at all times. The lighter an more active dog has a higher blood plasma volume per kilo and shows the greater daily fluctuations. These daily bleedings of 20 to 30 cc. cause a slight upset in the red blood cell hematocrit values and may be in part responsible for the variations in plasma volume figures.

SUMMARY

The *hemoglobin method* gives a reasonably accurate measure of the *circulating blood plasma.* Fresh standard hemoglobin solutions can be prepared easily and when used with sufficient care will give uniform figures which are identical with those obtained by the common vital dyes used in blood volume work. The hemoglobin is completely removed from the blood within 18 hours, which may be of advantage in certain experiments.

Observations here recorded show that large, fat dogs present a plasma volume per kilo which is uniformly lower than the figures for lean, active, adult dogs.

In our opinion this method gives no direct information concerning red cell or hemoglobin volume.

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BLOOD VOLUME STUDIES

VII. Comparative Values of Welcker, Carbon Monoxide and Dye Methods for Blood Volume Determinations. Accurate Estimation of Absolute Blood Volume

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Received for publication March 4, 1921

A review of the many blood volume methods and a consideration of the many apparent contradictions in these blood volume figures made it clear to us that a detailed study of these various methods was desirable. We hope to throw some light on many of these vexed questions so that finally we may come to understand the factors which regulate the volume of the circulating blood and recognize the limitations of the various methods. We submit the data of these experiments with the conviction that these figures obtained under uniform conditions, using the same animals and a controlled technic, are of greater value than the data to be obtained in the literature. The methods of different investigators are often incompletely described and may present gross errors—for example, the use of hypotonic oxalate solutions or dry powdered oxalate to prevent blood coagulation may introduce errors into the plasma volume figures which may exceed 10 per cent (1).

In brief, it is our conviction that the *dye or hemoglobin methods give the true plasma volume* (4.8 cc. per 100 grams of body weight), that the *carbon monoxide method gives the true body hemoglobin volume* (4.2 cc. R. B. C. per 100 grams body weight), and finally that the true blood volume is the sum of these two figures (9 cc. per 100 grams of body weight). Hematocrit figures introduce errors when used to calculate the plasma or red cell volume because of the assumption that the ratio of cells and plasma is a constant for all parts of the circulation. This error may become still larger in abnormal conditions where the ratio of cells to plasma may fluctuate more than normal in different areas of the circulation.

For several years these blood volume problems have been of interest to a considerable number of workers in this laboratory. These studies developed as a part of an investigation into the problem of hemoglobin regeneration after simple anemia. Some of these experiments have been published (2). In the course of this work certain defects in the blood volume method of Keith, Rowntree and Geraghty (3) were discovered and modifications were proposed by Hooper, Smith, Belt and Whipple (1). A variety of dye substances was tested by Dawson, Evans and Whipple (4). An acacia blood plasma volume method was described by McQuarrie and Davis (5) and certain advantages of this method pointed out. This critical study by many different workers of these various methods, their limitations and advantages, has been of great value in the development of an accurate and controlled technic.

In all of these methods the same principle is employed,viz., a known amount of the solution (dye, hemoglobin or acacia) is injected intravenously and adequate time is allowed for the complete admixture of this substance with the blood. At the end of this period a sample of blood is withdrawn into an isotonic oxalate solution and centrifugalized to remove the blood cells. The concentration of the injected substance in the supernatant plasma is then determined. It is now easy to compute the volume of plasma required to dilute to this degree the total mass of the substance injected. The original articles should be consulted for details of these methods. We wish to emphasize the fact that in theory all of these methods rest upon the same basis, viz., dilution of the injected substance by the blood *plasma*. For this reason these methods should be termed *plasma volume methods* rather than *blood volume methods*. A fact of equal importance is that these methods agree in showing that in normal adult dogs there are about 4.8 cc. of plasma per 100 grams of body weight.

The carbon monoxide method for the determination of blood volume has been the subject of study by Arnold, Carrier, Smith and Whipple (6). In its essential features their method is similar to that of Salvesen (7). They have carried out many controls on the method and have suggested some alterations in the method of Salvesen. The details of the method need not be repeated. In brief, the animal is forced to breathe into an air-tight but elastic chamber containing oxygen together with a measured amount of carbon monoxide. The carbon monoxide is drawn into the lungs along with the oxygen where it is absorbed almost quantitatively by the hemoglobin of the blood which passes continuously through the vessels of the lungs. The measured

amount of carbon monoxide is thus transferred from the respiratory chamber to the hemoglobin of the circulating blood. A sample of blood is now drawn and its carbon monoxide content determined. The carbon monoxide concentration in this sample furnishes an index of the total quantity of hemoglobin in the body. It will be noted that whereas the other methods mentioned above indicate the *volume of plasma*, the carbon monoxide is taken up into the hemoglobin and thereby furnishes us with an index of the *total body hemoglobin*.

For purposes of further comparison of methods we have used a modification of the direct blood volume method first employed in 1854 by Welcker (8). The method consists essentially in determining the amount of hemoglobin which can be washed out of the animal body. Various modifications and improvements of Welcker's method have been made in the last 50 years. Some of these changes have made possible the more complete removal of the hemoglobin from the body. Others involve the use of the more recently devised methods of estimating the amount of hemoglobin once it is washed out of the body. The method as used by us is described in detail below By means of this method, as in the carbon monoxide method, the blood constituent primarily measured is the hemoglobin. With certain qualifications to be mentioned later, either of these two methods enables one to determine fairly accurately the *total red blood cell volume*, for the hemoglobin of the circulating blood is normally confined entirely to the red blood cells and for each animal there is a definite ratio between the hemoglobin and the red blood cells. These two methods should be termed *body hemoglobin* or *red blood cell volume methods*.

In the experiments to be presented below, blood volume determinations were carried out on each of a series of normal dogs, first by the dye method, shortly thereafter by the carbon monoxide method, which was in turn followed by a determination by a modification of the Welcker method. In this way an excellent opportunity is afforded to study and compare these three methods under practically identical conditions of experimentation.

Method. Adult dogs maintained on a mixed diet were used in all experiments. All were sound, healthy animals unless otherwise noted. Free access to water obtained at all times. Food was withheld for a period of about 12 hours prior to the carrying out of the three blood volume determinations. This was done in order that the plasma might be clear and free from fat.

In carrying out the dye method the procedure previously outlined by Hooper, Smith, Belt and Whipple (1) was followed in all respects. Following this, usu-

ally within a few minutes, a determination by the carbon monoxide method as outlined by Arnold, Carrier, Smith and Whipple (6) was carried out. For each of these determinations approximately 20 cc. of blood were drawn. Preparations were now made for the determination by the third method, i.e., the modified Welcker method. The animal was placed under complete ether anesthesia. A cannula was inserted into one of the larger arteries (carotid or femoral) in such a manner that blood could be withdrawn at will. The animal was bled at a moderately rapid rate from this artery. In order to prolong life and permit the heart to pump out a major portion of the circulating blood cells, warm saline was injected into the vascular system through a cannula inserted into one of the larger veins. In all cases the saline was thus infused into either the femoral or external jugular vein. The fluid was injected at a rate approximately equal to that at which the blood was being withdrawn from the artery. In most cases the heart's action ceased after this exchange of saline for blood had been continued for about 20 minutes.

In order to wash from the vascular system as completely as possible the remaining traces of blood, the inflow cannula was changed and the saline was forced under pressure into the arterial system. The fluid thus artificially forced to circulate was in great part recovered from the right side of the heart by means of a cannula inserted and tied into the right auricular appendage in such a way as to drain the fluid collecting in the right auricle. In several of the earlier experiments a part of the saline forced into the arterial system found its way back past the aortic valves into the pulmonary circuit, and forcing its way into the bronchi escaped through the nose. In subsequent experiments this was prevented by clamping a heavy hemostat about the aorta just distal to the aortic cusps during the period of artificial perfusion. The perfusate was thus prevented from backing up into the pulmonary circulation.

During the period of perfusion the inflow of saline was occasionally interrupted for a number of seconds, for the work of Larson and Bell (9) indicates that intermittent perfusion is more efficacious in washing blood cells out of the vascular system than is a steady continuous flow. To further aid in adequately washing out of the smaller vessels general massage of the animal body was practiced. In most cases the artificial perfusion was continued for about 1½ hour. The fluid recovered during the last part of this period appeared almost as clear as water and on centrifugalization such samples were found to contain very few blood cells indeed. At autopsy only scattered traces of macroscopic blood could be made out. These findings are described more in detail in individual experiments. The relative freedom of the vessels from blood as shown by microscopic study of stained sections will also be shown later.

All samples of blood recovered from the animal were received into oxalate to prevent clotting. The blood withdrawn before the death of the animal was collected separately in one container. The blood collected by artificial perfusion was also collected separately, usually in two portions, the last portion being of relatively large volume (5 to 15 liters) but containing as a rule less than 10 per cent of the total hemoglobin recovered from the animals. Each sample thus collected was measured and its hemoglobin content determined by the method of Palmer (10). The total number of grams of hemoglobin removed was thus ascertained.

In spite of the prolonged artificial perfusion a certain small part of the total hemoglobin remains behind in the body. In order to measure the total amount of the pigment which perfusion failed to remove we have followed the practice of grinding up and extracting from the various organs and tissues their content of pigment. The skin in all cases was stripped off and being almost free of pigment was discarded. The skeletal muscles together with the fascia were dissected free from the skeletal system, ground up finely in a meat grinder, mixed with water and stirred frequently for a period of about 6 hours. The fluid and particles of muscle were separated in a meat press. A sample of the fluid obtained was further freed of debris by centrifugalization, after which its hemoglobin content was estimated. The parenchymatous organs were separately and individually treated in a similar manner. The gall-bladder with its contents was previously dissected free and discarded. The bile and fecal matter were carefully washed out of the gastro-intestinal tract before this tissue was ground up for extraction. The bones were broken up finely with a hatchet before their extraction was attempted. It is recognized that only a part of the total pigment thus extracted is true red blood cell pigment. Another portion is extravascular pigment, particularly muscle hemoglobin. For this reason the organ extracts are charted separately in all tables. The question as to how much of this pigment can properly be considered to be blood pigment will be discussed later.

The quantity of *blood hemoglobin* recovered by bleeding and perfusion is contained wholly within the red blood cells washed out. Knowing the total number of grams of hemoglobin, the total number of cubic centimeters of red blood cells can be learned. To do this the hemoglobin content of a given amount of red blood cells is determined by reading the hemoglobin content of an initial sample of blood from the same animal and taking into account the ratio of red blood cells to plasma (hematocrit). These figures give us a factor by which the total hemoglobin washed out may be multipled in order to show the total volume of red blood cells recovered. This same factor may be used to convert the hemoglobin figure into red blood cell volume in the case of the carbon monoxide method.

The hemoglobin and red blood cell volume figures are thus given in a very direct and relatively trustworthy manner by the carbon monoxide method or Welcker method. In like manner the dye method being essentially a plasma volume method indicates with fair accuracy the total volume of plasma. In tabulating our results we have placed in bold type those values for whose determination each method is especially adapted. This for the dye method includes plasma volume; for the carbon monoxide or Welcker methods, the total hemoglobin and red blood cell volume. All other figures tabulated are derived indirectly from these on the basis of the hematocrit. As will be explained later,

we have no assurance that the sample of blood centrifugalized (hematocrit) is representative of the entire blood. The figures based on the hematocrit are therefore open to criticism. Our method of tabulation enables one to distinguish these figures from those which are not open to this criticism and are placed in bold type.

Experimental observations. The general plan of experimentation has already been outlined. The results of 14 experiments are presented below. Several others which were incomplete in some important respect are omitted. Experiment 339 may be regarded as a typical experiment. The procedure followed is presented in detail as illustrative of the method used in all other experiments. In this experiment as in most of the others, glucose was added to the saline infused. Our experience indicates that if this be done the heart continues to beat for a longer time and a larger proportion of the blood is expelled before death occurs. This is to be desired because under conditions of artificial perfusion of dying tissues the vascular conditions are altered and circulation in the more remote parts of the vascular tree is sluggish. In addition the phenomenon of intravascular clotting following death may in some cases prove to be a disturbing factor. However, if artificial perfusion is relied upon to remove only the last traces of blood, such disturbing factors are of relatively slight importance. In this experiment the perfusion was continued for nearly 2 hours. During the greater part of this time the perfusate recovered was but slightly blood-tinged. During the last 30 minutes it was almost as clear and colorless as water. If 15 cc. of such a sample be centrifugalized only a very small layer of blood cells will be found in the bottom of the tube. In all 8 grams of hemoglobin were recovered through artificial perfusion, whereas 184 grams in all were recovered through bleeding and perfusion combined. Thus only 4.3 per cent of the total was recovered by perfusion.

Experiment 339. Dog 20–92. Adult male mongrel terrier, in good condition. Weight, 15.45 kgm. Blood volume determination by dye method at 7:37 a.m.: by the carbon monoxide method at 7:41 a.m. Ether anesthesia at 9:40 a.m. in preparation for carrying out the modified Welcker determination. A cannula was tied into the carotid artery. Bleeding from this cannula was commenced at 9:45 a.m. Simultaneously and at approximately the same rate Locke's solution containing 1 per cent glucose and warmed to body temperature was injected through a cannula into the external jugular vein. About 3500 cc. of this fluid were injected within a space of about 20 minutes. In the same period 3430 cc. of blood were withdrawn from the carotid artery. The heart continued to beat until nearly the end of this period at which time the blood flowing out was almost colorless. In order to wash from the vascular system as completely as possible

the remaining traces of blood, saline was forced under pressure into the arterial system by way of the carotid. The fluid thus artificially forced to circulate was recovered from the right side of the heart by means of a cannula inserted through the right auricular appendage into the right auricle of the heart. During the period of artificial perfusion the inflow of saline was occasionally interrupted for a period of a minute or so. To further aid the perfusate in reaching and adequately washing out all of the smaller vessels, general massage of the animal body was practiced. The perfusion was discontinued at 11:10 a.m. after having been in effect nearly 2 hours.

At autopsy the peritoneal and thoracic cavities contain a small amount of clear blood-tinged transudate. No blood is found in the cavities of the heart.

TABLE 15

Experiment 339. Dog. 15.45 kgm. Hematocrit 50.1 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc.*	*cc.*
Dye method	277.0	857.0	1710.0	838.0	1.79	5.55	11.07	5.42
Carbon monoxide method	223.0	690.0	1378.0	675.0	1.44	4.47	8.92	4.37
Welcker method								
Total bleedings	184.0	568.0	1133.0	555.0	1.19	3.68	7.33	3.59
Extracts								
Heart	0.23	0.70	1.40	0.69	0.0015	0.0045	0.0091	0.0045
Spleen	0.26	0.80	1.60	0.78	0.0017	0.0052	0.0104	0.0054
Skeletal muscles	22.8	71.0	141.0	69.0	0.147	0.459	0.913	0.447
Bones	3.32	10.3	21.0	10.0	0.021	0.067	0.133	0.065
Total extracts	26.6	83.0	165.0	80.0	0.171	0.536	1.066	0.522
Total (including extracts)	211.0	651.0	1298.0	635.0	1.36	4.22	8.40	4.11

Lungs, liver, kidneys, gastro-intestinal tissues contain only trace of hemoglobin.

The myocardium is pale brown. The lungs are crepitant throughout, are pale grey and quite bloodless. The spleen appears quite normal. The liver is uniformly brown. The lobulation is very indistinct. No blood visible anywhere. The mucosa of the esophagus is pink. The entire gastro-intestinal tract appears quite bloodless. The pancreas, kidneys and pelvic organs contain no macroscopic blood whatsoever. The bone marrow (femur, ribs) is of a moderate bright red color.

In microscopic sections no red blood cells are seen in the heart, lungs or liver, and only an occasional one can be found in the pancreas. The spleen is quite rich in red blood cells; none whatsoever are found in the kidneys or gastro-intestinal tract. No red blood cells could be found in the skeletal muscle even after prolonged search.

The hemoglobin content of the samples recovered was now determined. From the carotid artery 3430 cc. of blood were recovered before the death of the animal. This was found by the method of Palmer to contain 167 grams of hemoglobin. To this must be added 9 grams of hemoglobin which were contained in the 55.6 cc. of blood drawn as samples for the dye and carbon monoxide method. A total of 11,400 cc. was recovered during the period of artificial perfusion. In this there were 8 grams of hemoglobin. The sum of these quantities (184 grams) is entered in the table under the heading of "total bleedings." (See table 15.)

The skeletal muscles were stripped of and ground up finely in a meat grinder. Total weight, 6920 grams. This mass of ground muscle was thoroughly mixed up and to 500 grams 1500 cc. of distilled water were added. This was frequently and vigorously stirred for a period of several hours. By use of the meat press 1350 cc. of hemoglobin-tinged liquid were recovered. It is certain that there was more fluid which could not be forced out with the press. By a more powerful press we could undoubtedly have removed at least 1500 cc. (i.e., an amount corresponding to the quantity of water added for extraction). A sample of this fluid was further clarified by centrifugalization (30 minutes at 3000 revolutions a minute). To 100 cc. of this fluid 0.4 cc. of concentrated ammonia solution was added. Carbon monoxide was bubbled through and the solution was compared in a Duboscq colorimeter with a standard prepared as in the method of Palmer. This extract fluid was found to contain 0.11 gram of hemoglobin per 100 cc. At this rate 1500 cc. would contain 1.65 grams of hemoglobin. Had the entire 6920 grams of skeletal muscle been extracted instead of only 500 grams we should have recovered 22.8 grams of hemoglobin. The other organs and tissues were treated in the same manner. The bones were broken up finely with a hatchet before being extracted. In all cases the macerated tissues remaining behind in the meat press were quite pale, thus indicating that the extraction of hemoglobin had been quite complete.

A sample of blood drawn from the dog at 7:32 a.m. contained 50.1 per cent red blood cells as was shown by a thorough centrifugalization of a 10 cc. sample of blood drawn into a 15 cc. hematocrit tube containing 2 cc. of isotonic sodium oxalate (1.6 per cent (1)). Another sample of whole blood drawn at the same time was found to contain 16.20 grams of hemoglobin per 100 cc. Since the red blood cells constitute but 50.1 per cent of the volume of this blood it is easily seen that 100 cc. of packed cells would contain nearly twice this amount—32.3 grams per 100 cc., to be exact. This is equivalennt to saying that 1 gram of hemoglobin is contained in 3.09 cc. of red blood cells. At this rate 184 grams of hemoglobin (the amount in the "total bleedings") represent 568 cc. of red blood cells. Since there are 100 parts of blood for every 50.1 parts of red blood cells (as shown by the hematocrit) it follows that 568 cc. of red blood cells represent the quantity of red blood cells contained in 1133 cc. of blood. The difference between 1133 and 586 represents the volume of plasma plus that of the white blood cells. In this experiment the white blood cells comprised about 0.9 per cent of the total blood, as was shown by the hematocrit. The red blood cells plus the white blood cells, then, comprise 51.0 per cent of the blood. The other 49 per cent is plasma. Forty-nine per cent of 1133 is 555. This represents the total plasma in the "total bleedings." The organ extracts may be figured in the same manner with the results shown in table 15.

In the total extracts 26.6 grams more of hemoglobin were recovered. We are convinced that for practical purposes the pigment is completely extracted by our technic. A second extraction of the macerated bones was attempted in experiment 339, but as is noted in the protocol very little additional hemoglobin was dissolved out. Ordinarily a single extraction of finely macerated skeletal muscle leaves the muscle debris quite pale. Apparently by far the greater part of the pigment is removed. A second extraction of much muscle debris was made in a number of cases. A typical result is shown in experiment 349, table 16. In this case 15.9 grams were removed by the first extraction and but 2.0 grams by the second extraction. After the second extraction the muscle tissue was almost perfectly white. Similar extractions made in other organs and tissues show essentially identical results. Repeated extraction of such organs and tissues show that by far the greater portion of the pigment is removed by the first extraction, provided that to the macerated tissue at least an equal part of water be added for purposes of extraction.

Experiment 349. Dog 19–71. Adult male mongrel shepherd. For several weeks has been somewhat less active than normal and is somewhat undernourished but on the whole is in fairly good condition. Weight, 16.36 kgm. Blood volume determination by dye method at 8:40 a.m.; by the carbon monoxide method at 9:01 a.m. Bleeding and perfusion under ether anesthesia in the usual manner begun at 9:20 a.m. Warm 0.9 per cent saline to which was added 1 per cent of glucose was used for infusion. Following death of the animal the tissues were perfused with plain 0.9 per cent saline warmed to body temperature. A total of 24,580 cc. of blood-containing perfusion fluid was recovered.

At autopsy the thoracic organs appear to be almost perfectly free of blood. The spleen is normal in size and appearance. The liver is dark brown, the lobulation is distinct and no blood can be seen even at the centers of the lobules. The stomach appears to be bloodless. The mucosa of the upper part of the small intestine is faintly pink. The tips of some of the rugae of the mucosa of the large intestine are reddened. The pancreas, kidneys and pelvic organs are free of macroscopic blood. The bone marrow is bright red.

Microscopically but few red blood cells are seen in sections of myocardium. The lung contains a moderate number. As usual the spleen is quite rich in blood cells. A few are seen in the vessels in the hepatic spaces. Sections of pancreas are free of blood. A moderate number of red blood cells is found in the mucosa of the stomach and many in the mucosa of the small intestine. On the other hand sections of the large intestine and kidney are free of red blood cells. An occasional red blood cell is to be found in sections of skeletal muscle.

From the carotid artery 3580 cc. of blood were recovered before the death of the animal. This was found to contain 158 grams of hemoglobin. In addition to this 11 grams of hemoglobin were contained in the 83 cc. of blood drawn as samples. A total of 21,000 cc. of perfusate recovered during the period of arti-

ficial perfusion contained 22 grams of hemoglobin. The skeletal muscles (7136 grams) were ground up, mixed thoroughly and a 200-gram sample extracted in the usual manner with 400 cc. of water. After pressing out the fluid the muscle debris was quite pale. To this debris 200 cc. of water were added and the mixture frequently and thoroughly mixed for several hours. Had the entire mass of skeletal muscle been extracted instead of only 200 grams we should have obtained by the first extraction 15.9 grams of hemoglobin and only 2.0 grams by the second extraction.

The other organs were extracted in the usual manner. By the first extraction the bones yielded 4.8 grams of hemoglobin; by the second extraction, 0.8 gram.

TABLE 16

Experiment 349. Dog; 16.36 kgm. Hematocrit 46.4 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc.*	*cc.*
Dye method	218.0	822.0	1772.0	932.0	1.33	5.02	10.83	5.69
Carbon monoxide method	209.0	729.0	1571.0	826.0	1.28	4.46	9.60	5.05
Welcker method								
Total bleedings	191.0	664.0	1430.0	752.0	1.17	4.06	8.74	4.60
Extracts								
Heart	0.24	0.84	1.81	0.95	0.0015	0.0051	0.011	0.0058
Spleen	0.13	0.46	1.00	0.53	0.0008	0 0028	0.0061	0.0032
Skeletal muscles	15.9	55.0	119.0	63.0	0.097	0.34	0.73	0.39
Bones	4.8	17.0	36.0	19.0	0.029	0.10	0.22	0.12
Total extracts	21.1	73.0	158.0	83.0	0.128	0.45	0.97	0.52
Total (including extracts)	212.0	737.0	1588.0	835.0	1.30	4.51	9.71	5.12

Lungs, liver, kidneys and gastro-intestinal tissue contain only traces of hemoglobin.

Additional evidence of the readiness with which hemoglobin can be separated from macerated tissues is furnished by an experiment in which we added a known amount of hemoglobin (in the form of blood) to a weighed sample of macerated skeletal muscle. The mixture was thoroughly stirred after which its hemoglobin was extracted in the usual manner. Analysis showed that we recovered by a single extraction practically all of the added hemoglobin as well as the quantity of pigment which the muscle was known to contain before the artificial addition of hemoglobin.

The freedom of the perfused tissues from red blood cells as shown by microscopic study is well illustrated in experiment 339. Almost complete absence of red blood cells in all sections was noted. In sections from normal unperfused dogs many red blood cells are to be seen. Our experience has been that as a rule macroscopic appearance of the tissues as regards its blood content is well borne out by the microscopic findings. Experiment 351 also serves to illustrate this point. This experiment represents one in which we were particularly *unsuccessful* in perfusing the organs free of blood. The mucosa of the small intestine was brick-red in color and the abundance of red blood cells was quite strikingly shown in microscopic sections of this tissue. The large intestines were free of macroscopic blood and in confirmation no red blood cells whatever could be found microscopically. In this connection it will be recalled that in experiment 339 the absence of macroscopic blood in the entire gastro-intestinal tract was confirmed by microscopic study. The microscope furnishes, we believe, a rather delicate control on the completeness of the removal from the tissues of red blood cells. This is particularly fortunate since in certain tissues, such as skeletal muscle or myocardium, it is impossible to determine in gross just how much of the tissue color is due to residual blood and how much is due to pigments peculiar to the tissues themselves. It is important not to confuse the pigment normally contained within the muscle cells (muscle hemoglobin) with blood pigment. In none of our series was it possible to demonstrate more than a very occasional red blood cell in sections of skeletal muscle. In experiment 339, for instance, even after long-continued search, not a single red blood cell was found. It is hardly necessary to state that normal unperfused skeletal muscle shows considerable numbers of red blood cells. It would seem that the skeletal muscles were particularly easily freed of blood by perfusion. It must be that the conditions of circulation here are such that a free flow of saline to all of the finer ramifications of the blood vessels is quite readily accomplished. Curiously enough the kidney with its double capillary circulation is also left almost completely devoid of blood by perfusion. In contrast, other organs such as the gastro-intestinal tract can be thoroughly perfused only with difficulty. Still in most cases we have been able to remove the blood almost entirely. The spleen and bone marrow are unique in that they possess blood cells which are not in direct communication with the blood stream. A review of the experiments presented shows that red blood cells are made out in fairly large numbers in these tissues.

TABLE 17

Experiment 351. Dog; 9.00 kgm. Hematocrit 41.1 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc*	*cc.*
Dye method	119.0	381.0	928.0	538.0	1.32	4.23	10.31	5.98
Carbon monoxide method	111.0	358.0	871.0	505.0	1.23	3.98	9.68	5.61
Welcker method								
Total bleedings	95.0	306.0	744.0	432.0	1.06	3.40	8.27	4.80
Extracts								
Heart	0.14	0.45	1.10	0.64	0.0016	0.0050	0.0122	0.0071
Spleen	0.24	0.76	1.86	1.08	0.0027	0.0084	0.021	0.012
Liver	0.61	1.97	4.8	2.8	0.0068	0.022	0.053	0.031
Skeletal muscles	8.3	27.0	65.0	38.0	0.092	0.30	0.72	0.42
Total extracts	9.3	30.0	73.0	43.0	0.103	0.33	0.81	0.47
Total (including extracts)	104.0	336.0	817.0	475.0	1.16	3.73	9.08	5.27

Lungs, liver, kidneys and gastro-intestinal tissue contain only traces of hemoglobin.

Experiment 351. Dog 20–72. Short-haired female mongrel bull terrier. In good condition. Weight, 9.00 kgm. Blood volume determination by dye method at 8:30 a.m.; by the carbon monoxide method at 8:40 a.m. Bleeding and perfusion under ether anesthesia in the usual manner begun at 9:30 a.m. Total duration of perfusion was 2 hours. A total of 18,010 cc. of blood-containing fluid was recovered. The hemoglobin estimations were made in the usual manner.

At autopsy the myocardium is pale. The lungs are quite well perfused except for several small irregular red patches. The spleen is quite small but otherwise appears normal. In areas scattered irregularly over the surface of the liver blood can be seen at the centers of the lobules. On section the liver is pale grey and cloudy. The lobulation is quite indistinct. The stomach shows but the faintest traces of macroscopic blood. The mucosa of the entire small intestine is of a brick-red color. An excess of mucus is present. The large intestine appears to be bloodless. The pancreas is purplish grey and quite edematous. The kidneys and pelvic organs are free of macroscopic blood.

In microscopic sections no red blood cells are seen in the section of myocardium. Sections of lung show a few red blood cells. Blood cells are very numerous in the spleen. The liver is cloudy. Its lobulation is poor. No red blood cells are seen. The villi of the small intestine are quite rich in red blood cells, although but few are to be seen deeper in the mucosa. No signs of blood seen in sections of the large intestine, kidney or skeletal muscle. A moderate number of red blood cells is seen in the pancreas.

The organs and tissues were extracted in the usual manner (see table 17).

In table 18 the quantity of hemoglobin extracted from the various organs is charted. The averages agree fairly well with the findings in the experiments already presented. The amount of hemoglobin is expressed in terms of body weight. It was impossible in this series to express the hemoglobin from each organ in terms of the total weight of that organ on account of the fact that most of the organs were quite edematous from perfusion and it was impossible to gain any very accurate notion of the correct weights of these organs. However, we have noted the weights of the various organs from other dogs killed for other reasons. Assuming that the same figures hold for this series of dogs we may calculate from table 18 that the lungs (normal weight 115 grams) must have contained about 0.01 gram of hemoglobin per 100 grams of tissue. The gastro-intestinal tract (normal weight about 500 grams) being as a rule somewhat less perfectly perfused, contained 0.015 gram per 100 grams of tissue. The liver (average weight about 420 grams) contains about 0.013 gram per 100 grams of liver substance. The spleen which is so rich in residual blood contains about 0.10 gram per 100 grams of tissue or approximately 0.25 gram for the entire organ. This same table shows that the organ extracts have a total of 0.126 gram of hemoglobin per 100 grams of body weight. Of this 0.103 gram or 82 per cent of the total is derived from the skeletal muscles. This seems rather high when it is recalled that microscopic study showed the muscles to contain so few red blood cells. Furthermore, using the results of table 18 as a basis it may be computed that from each 100 grams of skeletal muscle 0.02 gram of hemoglobin was extracted. This is little more than was recovered from a like quantity of lung tissue, but much less than was recovered from the same amount of splenic tissue. Still when it is recalled that the skeletal muscles constitute nearly one-half of the total body weight it will be seen that the total amount of pigment so recovered must of necessity constitute a large part of the total extracts. The relatively large amount of hemoglobin removed from the skeletal muscles by extraction must be derived only to a small extent from blood contained in this tissue. There can be little doubt that the greater part of this pigment is not blood hemoglobin but muscle hemoglobin. The existence within the striped muscle of such a pigment is well known. It has been shown that it is very similar to, if not identical with, blood hemoglobin (11).

From the bones 0.020 gram of hemoglobin per 100 grams of body weight was recovered. Much of this likewise cannot be considered as belonging to the circulating blood. Part of this exists in extravascular

TABLE 18

Summary of hemoglobin values in Welcker experiments

EXPERIMENT	WEIGHT OF DOG	HEMATOCRIT (R. B. C.)	HEMOGLOBIN PER 100 GRAMS OF BODY WEIGHT RECOVERED FROM									
			Total bleedings plus perfusions	Organ extracts								Total bleedings plus perfusions and extracts
				Heart	Lungs	Spleen	Liver	Stomach and intestines	Skeletal muscles	Bones	Total extracts	
	kgm.	*per cent*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
330	4.75	45.3	1.26			0.0044	0.0063	0.0103	0.101	0.033	0.160	1.42
356	8.77	39.8	0.88	0.0024	0.0013	0.0027			0.11	0.025	0.14	1.02
335	8.86	46.2	0.77	0.0015		0.0008	0.0031		0.087	0.005	0.097	0.87
351	9.00	41.1	1.06	0.0016		0.0027	0.0068		0.092		0.103	1.16
333	9.18	53.3	1.24	0.0031		0.0039			0.099	0.027	0.133	1.37
340	10.45	52.1	0.93	0.0015		0.0016			0.146	0.015	0.164	1.09
328	10.46	48.8	1.19		0.0011	0.0021			0.104		0.107	1.30
346	10.68	62.0	1.17	0.0010		0.0014			0.070	0.021	0.093	1.26
342	11.82	48.3	1.18	0.0015		0.0015			0.113	0.015	0.131	1.31
344	13.64	58.9	1.30	0.0014	0.0007	0.0018			0.075	0.018	0.097	1.40
345	13.64	59.5	1.34	0.0013		0.0012			0.094	0.018	0.114	1.45
339	15.45	50.1	1.19	0.0015		0.0017			0.147	0.021	0.171	1.36
349	16.36	46.4	1.17	0.0015		0.0008			0.097	0.029	0.128	1.30
343	18.64	58.0	1.39	0.0006	0.0006	0.0021	0.0018	0.0016	0.102	0.012	0.121	1.51
Average..	11.55	50.7	1.14	0.0016	0.0009	0.0021	0.0045	0.0060	0.103	0.020	0.126	1.27

bone marrow cells which have not yet matured and been thrown into circulation. The spleen contains many red blood cells which are not in active circulation. These cannot be removed by any amount of perfusion. The liver is of a brownish tint due to pigments peculiar to the liver. The presence of these pigments makes it difficult to estimate colorimetrically its blood hemoglobin content unless the latter be fairly large.

In many experiments some of the organs were so well perfused that by extraction such small amounts of hemoglobin were recovered that their quantitative estimation was impossible. In these cases no figures at all are entered in the tables. For this reason the figure for total extracts is slightly smaller than it would be if it had been possible to include the extracts of all organs.

In view of all of these facts obviously considerable difficulty surrounds an attempt to state with any degree of certainty just how much of the total hemoglobin extracted should be considered to be derived from blood cells left behind in the vessels after completion of perfusion. It would seem that the blood hemoglobin must constitute somewhat less than one-half of the total pigment extracted. If, however, we add a small amount for incomplete extraction of the tissues we will probably not err greatly in assuming such a figure. One-half of the total extracts would be 0.063 gram of hemoglobin per 100 grams of body weight. If this amount of hemoglobin be added to that obtained by bleeding and perfusion combined (1.14 grams per 100 grams of body weight) we have a total of 1.20 grams of *blood hemoglobin* per 100 grams of body weight. Even though our assumption that one-half of the pigment extracted is blood hemoglobin is somewhat erroneous, still this figure of 1.20 will be but slightly affected, for a difference of only 5 per cent results, if we neglect the extracts altogether or if we assume the other extreme—viz., that *all* of the extracted pigment is blood hemoglobin.

Muscle hemoglobin and other extravascular pigments of similar nature furnish subject for debate not only in the case of the Welcker method but in the carbon monoxide method as well. The muscle hemoglobin has been shown (11) to be very similar to that of blood hemoglobin and on being placed in contact with carbon monoxide it unites with the latter to form a substance having a spectrum similar to that of the carbon monoxide hemoglobin derived from blood hemoglobin. It seems quite probable that the carbon monoxide, which is administered in the carbon monoxide method and is taken up by the red blood cells, must through processes of diffusion reach and combine to some extent

TABLE 19

Summary of blood volume and hemoglobin values

EXPERIMENT	WEIGHT OF DOG	HEMATOCRIT (R.B.C.)	PER 100 GRAMS BODY WEIGHT														
			Hemoglobin				Red blood cells				Whole blood				Plasma		
			Dye method	CO method	Welcker method		Dye method	CO method	Welcker method		Dye method	CO method	Welcker method		Dye method	CO method	Welcker method
					With extracts	Without extracts			With extracts	Without extracts			With extracts	Without extracts			With extracts
	kgm.	*per cent*	*grams*	*grams*	*grams*	*grams*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
330	4.75	45.3	2.11	1.96	1.42	1.26	5.68	5.28	3.83	3.41	12.53	11.68	8.48	7.52	6.74	6.29	4.55
356	8.77	39.8	1.14	1.03	1.02	0.88	3.64	3.30	3.22	2.78	9.14	8.27	8.12	7.00	5.30	4.80	4.70
335	8.86	46.2	1.24	0.76	0.87	0.77	4.66	2.84	3.26	2.89	10.08	6.15	7.03	6.24	5.27	3.22	3.67
351	9.00	41.1	1.32	1.23	1.16	1.06	4.23	3.98	3.73	3.40	10.31	9.68	9.08	8.27	5.98	5.61	5.27
333	9.18	53.3	1.42	1.67	1.37	1.24	4.79	5.65	4.65	4.19	8.99	10.60	8.71	7.86	4.10	4.84	3.97
340	10.45	52.1	1.51	1.13	1.09	0.93	5.08	3.79	3.67	3.13	9.75	7.27	7.06	6.01	4.58	3.41	3.32
328	10.46	48.8	1.52	1.20	1.30	1.19	5.15	4.07	4.38	4.02	10.55	8.34	8.97	8.22	5.28	4.17	4.48
346	10.68	62.0	1.58	1.42	1.26	1.17	6.16	5.53	4.90	4.54	9.94	8.93	7.90	7.32	3.70	3.32	2.93
342	11.82	48.3	1.79	1.36	1.31	1.18	5.58	4.25	4.09	3.67	11.57	8.79	8.45	7.60	5.76	4.37	4.20
344	13.64	58.9	1.97	1.44	1.40	1.30	6.59	4.82	4.69	4.36	11.19	8.18	7.96	7.41	4.51	3.30	3.20
345	13.64	59.5	1.69	1.35	1.45	1.34	5.78	4.62	4.98	4.59	9.72	7.76	8.34	7.68	3.85	3.07	3.31
339	15.45	50.1	1.79	1.44	1.36	1.19	5.55	4.47	4.22	3.68	11.07	8.92	8.40	7.33	5.42	4.37	4.11
349	16.36	46.4	1.33	1.28	1.30	1.17	5.02	4.46	4.51	4.06	10.83	9.60	9.71	8.74	5.69	5.05	5.12
343	18.64	58.0	2.08	1.51	1.51	1.39	6.04	4.37	4.41	4.05	10.42	7.53	7.60	6.98	4.29	3.10	3.13
Average	11.55	50.7	1.61	1.34	1.27	1.15	5.28	4.39	4.18	3.77	10.39	8.69	8.27	7.44	5.03	4.21	4.00

with the muscle hemoglobin. It seems more than likely that the carbon monoxide method indicates not only the blood hemoglobin but takes in the muscle hemoglobin as well. We know of no other compound in the mammalian body which possesses an especial affinity for carbon monoxide. By physical solution at the carbon monoxide tension obtaining in the plasma of blood one-half saturated with carbon monoxide not more than 2 or 3 cc. of this gas could be taken up by all of the body fluids, even assuming the latter to constitute a very great proportion of the body. The error arising from this source would not exceed 2 or 3 per cent. It is improbable that this amount of CO could be held in body fluids in the presence of unsaturated hemoglobin.

Further study of table 19 shows in general the same figures for hemoglobin and red cells by the CO method and the Welcker method. The average figures show that the CO method gives figures which are 5 per cent greater than those recorded by the Welcker method for hemoglobin and tissue extracts combined. In general the figures correspond within reasonable limits. It is more simple to explain the occasional higher Welcker figures because we can assume incomplete equilibrium and mixing of the circulating CO. When the CO figures are higher than the Welcker figures we must assume some limitations in the accuracy of the technical procedure. The first experiment in table 19 (no. 330) is the only one which shows a startling discrepancy between the CO and Welcker figures. We have observed in other experiments that small or young dogs apparently had an unusual capacity of removal of CO from the blood stream. This point is being investigated further. Other than this the probability of a technical error must be considered as the figure of 5.28 cc. per 100 grams body weight is much above the average of 4.39. We see, however, that individual variations in this group of dogs is quite considerable—5.65 cc. per 100 grams, a maximum and 2.84 cc. per 100 grams, a minimum—average of 4.39 cc. This shows 100 per cent fluctuation between minimum and maximum. We believe that the accumulation of more data will show that the larger dogs show less variation in blood volume factors. These variations are not associated with anemia as shown by the hematocrit column. The plasma volume figures show less notable fluctuations—a maximum of 6.74 cc. per 100 grams body weight—a minimum of 3.70 cc. and an average of 5.03 cc.

From table 19 it is seen that 1.34 grams of hemoglobin per 100 grams of body weight are found by the carbon monoxide method. This is considerably higher than the amount which could be recovered in the

Welcker method by bleeding and perfusion combined. If, however, the total extracts are included in the Welcker determination we have a figure which approximates the value of 1.34 obtained by the carbon monoxide method. If experimental errors be taken into account these two values may be considered as almost identical. That this total hemoglobin (blood plus muscle hemoglobin) of the Welcker method should approximate the total hemoglobin, as determined by the carbon monoxide method, is evidence in favor of the view just outlined that by the carbon monoxide method the muscle hemoglobin as well as the blood hemoglobin is estimated.

DISCUSSION

Before attempting further to compare and correlate these three blood volume methods let us emphasize what has already been stated, viz., that the values *directly* obtained by each of the methods are in all cases tabulated in bold type. These figures include for the dye method the plasma volume; for the carbon monoxide and Welcker methods, the figures for hemoglobin and red blood cell volume. All other figures are derived *indirectly* from these figures on the *assumption* that the red blood cell volume bears a ratio to the plasma volume identical to the ratio of reds to plasma observed in a centrifugalized sample of blood drawn from the jugular vein. In this assumption is involved the idea that the plasma and red blood cells are thoroughly and uniformly mixed within the vascular system and that a sample of blood taken from the jugular vein contains neither more nor less than its proper proportion of red blood cells. But this assumption falls to the ground if it can be shown that conditions in certain parts of the vascular system (e. g., certain of the smaller vessels) are such as to favor undue retention of either plasma or red blood cells. We shall discuss presently certain evidence in favor of the view that the blood is far from being uniformly mixed. At any rate while the assumption mentioned above is under suspicion it is well to trust only the figures placed in bold type. Most previous investigators have unreservedly accepted the other figures also. These figures are included in our charts so that our results may be conveniently compared with and correlated with the results of previous workers.

We believe that the evidence already presented entitles one to conclude that the carbon monoxide method or the Welcker method furnished fairly reliable data concerning the total red blood cell volume. As has been noted above the maximum figures by either method in all

probability represent a figure which is somewhat greater than the true red blood cell volume. For in the Welcker method a part of the pigment extracted is not blood hemoglobin, but muscle hemoglobin. This muscle hemoglobin probably unites with carbon monoxide and thereby causes the carbon monoxide method to give values that are equally too high. If we deduct from the results of both methods an amount equal to one-half of the total organ extracts we shall have a red cell volume of 4.18 cc. per 100 grams of body weight by the carbon monoxide method and 3.97 by the Welcker method (see table 19). At any rate in round numbers 4.1 cc. per 100 grams must represent a very close approximation to the true red blood cell volume.

It must be emphasized repeatedly that the volume of *actively circulating red cells* is definitely less than the total *body hemoglobin* which, of course, includes muscle hemoglobin (Myohematin) and red cells in stagnant sinusoids in spleen and red marrow. It appears probable that this *immobile hemoglobin* represents 5 to 10 per cent of the total body hemoglobin which is concerned in the dilution of inhaled carbon monoxide. The CO method does not permit us to differentiate between the rapidly circulating hemoglobin and the immobile or fixed hemoglobin. Perhaps some method may be devised by which this difficulty can be surmounted.

The proof as to the size of the *plasma volume* is somewhat more difficult to obtain. To summarize, there are many methods for the determination of plasma volume. In all of them the principle is the same as the principle of the dye method. Dyes other than vital red have been used. Acacia, gelatin, dextrose, antitoxin and hemoglobin may also be substituted for the dye. All of these methods agree in indicating that the plasma volume is of the magnitude stated (4.8 cc. per 100 grams of body weight). It is difficult to believe that all of the substances thus used for the determination of plasma volume would be eliminated to any great extent in the 4-minute period allowed for their admixture with the plasma. Especially difficult is it to believe that substances so diverse in nature would all be so much more rapidly eliminated in the first few seconds than thereafter. The fact that dye once introduced can be recovered in large measure by perfusion indicates that destruction or elimination of dye is not great. Furthermore, the elimination of dye from circulation may be studied by withdrawing samples of blood for analysis at varying intervals of time following injection of the dye. A curve may be plotted to indicate these values. If this curve be drawn backward to cover the period prior to the taking of the 4-minute sample the result indicates that not more than 5 per cent

of the total dye injected is eliminated within the first 4 minutes. If a correction be made for this, the method shows that there are 4.8 cc. of plasma per 100 grams of body weight. We hope very soon to present experimental data to supplement this brief review of the plasma volume factors.

By *blood volume* is meant the sum *total of plasma volume, total red blood cell volume* and *white blood cell volume.* We have just discussed a method which determines plasma volume (dye method) and two others (carbon monoxide, Welcker) which determine red blood cell volume. No thoroughly reliable data concerning the total volume of white blood cells exists. Our hematocrit figures indicate that on an average the white blood cells constitute about 1.3 per cent by volume of the circulating blood. The work of Brodin, Richet and Saint-Girons (12) indicates that only about 50 per cent of all the white blood cells are in active circulation, the others being retained presumably in some of the smaller vessels. On the basis of these observations the white blood cell volume must constitute about 2.3 per cent of the total blood. If so, there are approximately 0.21 cc. of white blood cells per 100 grams of body weight. In this case the blood volume would equal per 100 grams of body weight, 4.8 cc. of plasma plus 4.2 cc. of red blood cells plus 0.2 cc. of white blood cells—a total of 9.2 cc. This we feel represents fairly accurately the total blood volume in our series of dogs. We wish to emphaszie our belief that it is only by a combination of methods that a proper estimate of the blood volume can be obtained. There is no justification for the custom of assuming that the plasma volume and red blood cell volume bear to each other the same ratio as is found in a centrifugalized sample of blood drawn from any of the usual sources. Yet this is exactly what is done when by the use of the hematocrit the total red blood cell volume is derived on the basis of a plasma volume previously determined by the dye method or the acacia method or any of the other plasma volume methods. It is equally incorrect to attempt to estimate the plasma volume when merely the red blood cell volume has been determined by either the carbon monoxide method or the method of Welcker. Such estimations involve the assumption that the ratio of red blood cells to plasma in blood drawn from the jugular vein is a ratio which is representative of the blood as a whole. A great amount of work has been reported which indicates that the distribution of blood cells in the body is a very changeable factor. The well-known observations of Cohnstein and Zuntz (13) may be cited in this regard. By microscopic study of living preparations they found that the smaller

vessels became crowded with red blood cells at the expense of plasma under certain conditions of vascular relaxation. Under conditions of vasoconstriction the red blood cells became less numerous and these fine vessels contained relatively more plasma. Much of the work on red blood cell count changes with high altitude and with exercise and the like may be explained on the basis of altered distribution of the blood cells. Confirmation of such theories is lacking, for as yet no investigator has studied these problems with a combination of a plasma volume method and a red blood cell method. The mere fact that while the red blood cell count rises, the red blood cell volume (or the total oxygen capacity of the blood) is unaltered does not indicate what changes may be occurring in the total volume of plasma. The work of Lamson shows that under certain conditions the blood cells may tend to accumulate in the liver (14).

We believe that the results of the present paper indicate in a way hitherto unsuspected, the variable admixture of the plasma and red blood cells. A review of table 19 shows that on an average there are 5.03 cc. of plasma per 100 grams of body weight. As has been already explained it seems probable that about 4 or 5 per cent of the dye injected is eliminated in the 4 minutes allowed for mixing of the dye with the plasma. Granting that this is true the corrected plasma volume is about 4.8 cc. of plasma per 100 grams of body weight. According to the same table there are about 4.2 cc. of red blood cells per 100 grams of body weight. The plasma together with the red blood cells equals the sum of these two figures or 9.0 cc. per 100 grams of body weight. If to this the probable white blood cell volume (0.2 cc. per 100 grams of body weight) be added, a sum-total of 9.2 cc. per 100 grams of body weight is obtained. This represents, we believe, a close approximation of the total blood volume. Since the red blood cells constitute 4.2 cc. of this 9.2 cc. it is easily seen that the red blood cells constitute 45.5 per cent of the total blood. This value represents the true red blood cell hematocrit. If, for instance, all of the plasma and blood cells could be removed from the body, placed in a large container and completely mixed, a sample of this blood on centrifugalization would be found to contain 45.5 per cent of red blood cells. In the living animal it is impossible to secure any such ideal sample, since it is impossible to take blood from all parts of the vascular system at once. A sample of blood taken from the jugular vein contains 50.7 per cent of red blood cells (table 19)—a figure considerably higher than the true 45.5 per cent. It would seem then that blood taken from the jugular vein is consider-

ably too rich in red blood cells. In an effort to locate other regions having a corresponding poverty in red blood cells we have drawn samples from the femoral vein, carotid artery, femoral artery, portal vein and other large vessels. Needless to say obstruction to the flow during the taking of all such samples was carefully avoided. It is not necessary to present all of the figures in detail. It will suffice to say that blood drawn from all of these sources was found within very narrow limits to contain the same percentage of red blood cells as was found in blood drawn from the jugular vein. We next turned our attention to the smaller vessels. It is very difficult to obtain any very exact information regarding the richness in red blood cells of the blood contained in such vessels. However, certain microscopic findings enable us to draw some general conclusions. Almost as soon as the microscopic circulation of the blood was first discovered it was observed that the flow in the smaller vessels was not at a uniform rate in all parts of the cross section. It was noted that in the central or axial portion of the stream the flow is much more rapid than in the peripheral portion of the stream. Furthermore the red blood cells are to be seen almost exclusively in the central rapidly moving part of the stream. If the laws governing the flow of simple liquids in tubes hold here, the center of the stream moves at a rate equal to twice the average velocity of the stream. The "still space" represents a body of plasma which is relatively stagnant—withdrawn from the circulation, as it were. The width of this layer undoubtedly varies in different vessels and under different conditions of flow. It is ordinarily stated that this layer has a width of at least one-eighth of the diameter of the vessel. If this be so it can easily be shown that this "still space" constitutes about 44 per cent of the cross sectional area of the vessel. Of course the plasma in this "still space" is not perfectly stationary and hence is not completely withdrawn from circulation. The rate of movement in any part of the vessel is approximately inversely proportional to its distance from the center. At this rate the plasma in the inner fourth of this so-called "still space" may be thought of as moving at the same rate as the innermost or "axial core" while the outer three-fourths of the "still space (i. e., the three-fourths nearest the vessel wall) may then be considered as being perfectly stationary. If, then, the "still space" constitutes 44 per cent of the cross sectional area of the vessel and three-fourths of this "still space" can be considered as being stationary it follows that 33 per cent (75 per cent of 44 per cent) of the contents of the vessel is stagnant plasma. The other 67 per cent of the content

of these small vessels is the actively circulating blood of the body and in our series of animals contained 50.7 per cent by volume of red blood cells. On an average there were 4.1 cc. of red blood cells per 100 grams of body weight. If these cells were all actively circulating our hematocrit would indicate that they should be accompanied by 4.0 cc. per 100 grams of body weight of actively circulating plasma. Our experiments show that the sum total of plasma is about 4.8 cc. per 100 grams of body weight. The difference between 4.8 and 4.0 is 0.8—a figure which represents the number of cubic centimeters of stagnant plasma per 100 grams of body weight. If the true blood volume be 9.0 cc. per 100 grams of body weight it is seen that the stagnant plasma constitutes about 9 per cent by volume of the total blood. All of this plasma could be stored away in the "still spaces" of the smaller vessels on the assumption that such small vessels contained one-third of the total blood. For, as was estimated above, about 33 per cent of the content of the smaller vessels consists of stagnant plasma for which we must account. It does not seem unreasonable to believe that one-third of the total blood is contained in the small vessels possessing an axial stream, particularly when it is recalled that the total cross section of the finer arterioles and venules has been estimated to be several hundred times the cross section of the aorta. Furthermore, the length of some of the arterioles is considerable. The total combined content of all such vessels must be very great.

Unfortunately previous microscopic studies and measurements do not enable us to establish conclusively our theory that the total volume of the "still space" is great enough to account for all of the plasma which we have shown is stored somewhere. We hope within the near future to be able to collect definite first-hand data on this point. These considerations do, however, establish the fact that there is within the "still spaces" at least a considerable quantity of stagnant plasma.

We must not overlook the possibility that plasma may also be "stored" in other regions. Thus Cohnstein and Zuntz (13) held that there are capillaries which are filled with plasma to the exclusion of red blood cells. Some investigators may wish to maintain that a certain part of the extravascular lymph spaces are quite accessible to the dye and that the lymph constitutes some of the stagnant part of our "plasma volume." The evidence now at hand is somewhat against this view. Our theory involving the "still spaces" seems to cover all known facts both from a qualitative and quantitative standpoint. This view in no way conflicts with the work of those who wish to maintain that under certain

conditions of circulation, red blood cells may be stored or deposited to excess in certain parts of the vascular tree. Nor do we wish to deny that under certain conditions the plasma volume may vary to a considerable degree. Further evidence is being collected on these points.

We wish to emphasize the view already presented to explain why the total "blood volume" as obtained by the dye method constitutes 9.5 to 10.5 cc. per 100 grams of body weight, while the total "blood volume" obtained by the carbon monoxide or Welcker method constitutes but 8 cc. per 100 grams of body weight. For blood drawn from the jugular vein is composed of approximately equal parts of plasma and blood cells. Anyone who did not know of the plasma stored away in the smaller vessels might make the mistake of thinking that the blood volume was twice the plasma volume or twice the total blood cell volume. In case he had already established by the dye method the fact that there are 4.8 cc. of plasma per 100 grams of body weight, he would be led to conclude there there is twice this amount of blood i. e., 9.6 cc. of blood per 100 grams of body weight. On the other hand, if he had established by the carbon monoxide or Welcker method that the red blood cell volume is 4.1 cc. per 100 grams of body weight he would conclude that there are but 8.2 cc. of blood per 100 grams. As our discussion shows, such calculations are not based upon sound premises. These workers should combine the plasma volume obtained by the dye method with the red blood cell volume obtained by the carbon monoxide or Welcker method. The result would be 8.9 cc. per 100 grams of body weight. If desired, one might add 0.2 cc. for white blood cells. In this case the observer would conclude that there are 9.1 cc. of blood per 100 grams of body weight. This, we believe, represents a correct approximation of the actual blood volume.

SUMMARY

Three standard blood volume methods are compared by the same workers under uniform conditions with a carefully controlled technic. The dye method, the carbon monoxide method and a modified Welcker method are all used on the same dog within a short space of time. The comparative value of these figures is thereby enhanced.

The dye method determines with reasonable accuracy the plasma volume but not the red cell volume. The carbon monoxide or Welcker method determines the total hemoglobin or red cell volume but not the plasma volume.

The dye method in normal dogs gives a plasma volume of about 4.8 cc. of plasma per 100 grams body weight. The carbon monoxide method in normal dogs gives the red cell volume as 4.2 cc. per 100 grams body weight. There are probably about 0.2 cc. of white blood cells per 100 grams body weight. In our opinion the *correct blood volume* is the sum of these figures or 4.8 cc. plasma + 4.2 cc. red cells + 0.2 cc. white blood cells = *9.2 cc. blood volume* per 100 grams body weight.

We believe other investigators have fallen into error due to *calculation of the total blood volume* based on the red cell hematocrit—usually 50 per cent in normal dogs. This gives erroneous figures of about 10 cc. per 100 grams body weight for the dye method and about 8 cc. per 100 grams body weight for the carbon monoxide method.

This error comes from the assumption that the blood cells and plasma are uniformly mixed in all parts of the circulating system. There is sufficient evidence to point to an *excess of plasma* over red cells in arterioles and capillaries. The axial cell stream in arterioles, for example, gives a marginal "still space" in which a considerable amount of plasma may be stored.

The absolute value of circulating red cells must always be somewhat smaller than the carbon monoxide figures which express the total *body hemoglobin*, inclusive of muscle hemoglobin and hemoglobin in red marrow and spleen sinusoids. The amount of this *immobile body hemoglobin* is about 5 to 10 per cent of the total body hemoglobin.

The true *total blood volume* can be obtained by the use of the carbon monoxide method (red cell volume) plus the dye or similar method (plasma volume). The sum of these figures gives the true blood volume.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

IV. The Maintenance of a Practically Uniform Temperature in Rabbits by the Elimination of Random Movements

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Received for publication March 8, 1921

In a previous paper (1) I reported the normal temperature variations in rabbits and the temperature effects of operative procedures. The animals were kept in large boxes in the experimental room in order to avoid the disturbing effect of daily removal from the animal house. They were always handled so as to cause as little excitement as possible and were fed daily about 6 p.m., that is, the rabbits were kept uniformly undisturbed but were allowed freedom in moving at will about the case (2 × 3 feet square). They thus generally indulged in moderate exercise.

Curve *A* is a specimen of the daily temperature variations of two rabbits under the above conditions, showing ranges of 0.7°–1.4°C. in the course of the day. In some cases the range was much greater, one varying 2.6°C. in one day. "The figures and curves show that one temperature observation cannot be used as the norm for that rabbit. A change above or below this one reading cannot be considered to be experimentally produced unless it is great enough to fall beyond the range of normal variability. Some workers consider a steady rise or fall as experimental compared with the fluctuating normal. It is conceivable that the range would need to be determined for any given experimental environment" (1).

In the same paper temperature variations were also given for rabbits which, for experimental purposes, have been bound back uppermost to an operating board. Under these conditions a marked fall, 1–2°C. or more, was recorded, the temperature remaining constant after the low level had been reached. This constant level was, however, too low to be used as a basis for experimental changes.

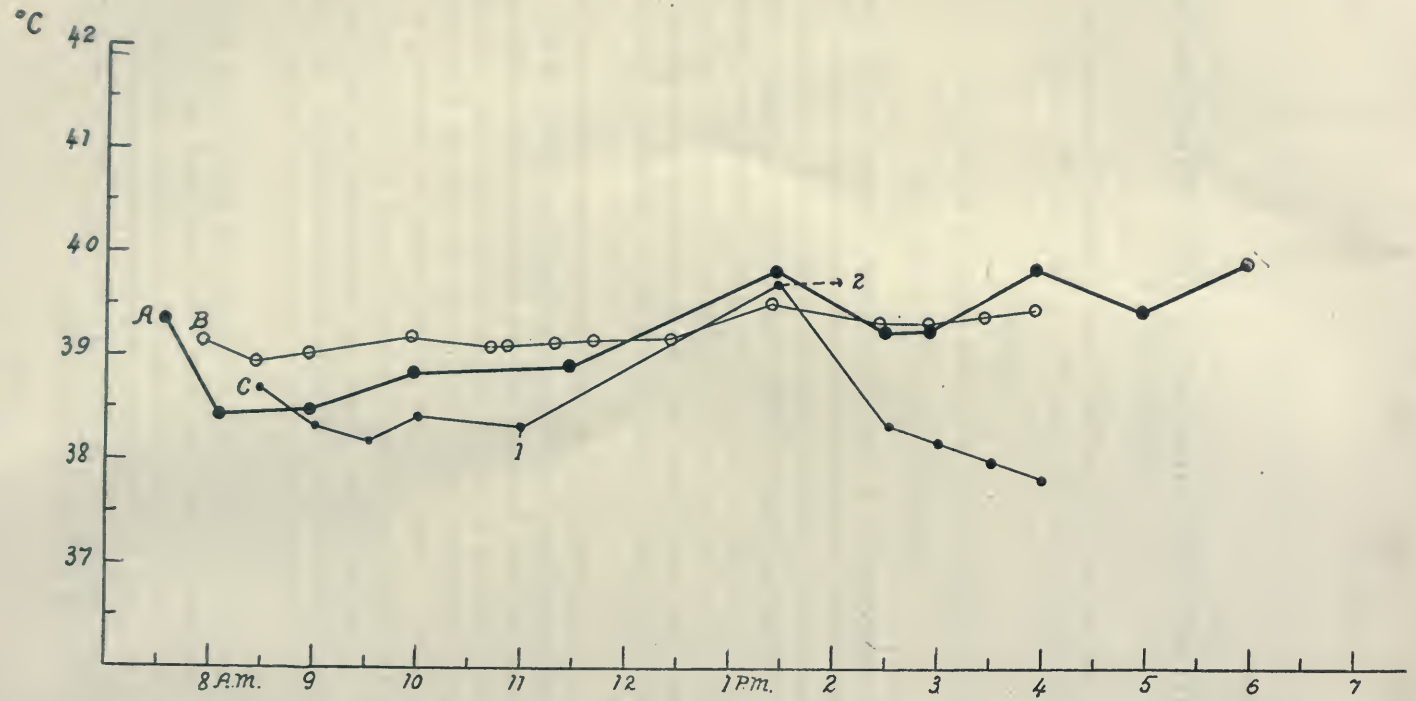

Fig. 1. *A.*—Normal temperature curve of rabbit in cage. *B.*—Normal temperature curve of rabbit in box. *C.*—Normal temperature curve of rabbit in box from beginning to *1*; loose in cage from *1–2*; in box from *2* to end.

The marked normal temperature variations indicated above tend to make the rabbit an unsatisfactory animal for the experimental studies of the regulation of body temperature. Further attempts were made, however, to establish an experimental environment which would result in a more constant temperature norm with less chance of fortuitous extremes. The result of this effort is the basis of the present paper.

Rabbits instead of being allowed freedom in a large cage were placed in a small box 6 × 18 inches long and 8 inches deep, in which they generally remained quiescent for hours. To insure absolute quiet, the head was placed in a Zwarmak head holder in one end of the box. In this position they appeared to be very contented and comfortable after the preliminary adjustments and would remain for hours without even an attempt at movement. There were exceptions, to be sure. Occasionally a male rabbit would make violent effort to escape. Those remaining quiet were assured of a less fluctuating temperature by the elimination of the effects of muscular exercise. Thus, one very pronounced variable which might easily be responsible for the wide range of normal variability was controlled, leaving only such as depend on food, sex, age, etc., all of minor importance. The pyretic effect of muscular exercise is a matter of every-day observation. Experimental exercise in rabbits has been reported by Krause (2) to cause a rise from 39.05°C. to 40.1°C. in 7 minutes. Thus chance movements, even though slight, would be expected to produce temperature changes of sufficient magnitude to mask many of experimental origin.

Curves of the temperature variations after the effects of muscular exercise have been eliminated show an almost constant temperature after the preliminary fall which marks the recovery from previous movement or that accompanying the process of adjustment in the box. The only apparent change in most cases was a gradual slight rise toward the end of the day. Marked fluctuations were never observed. In a number of cases the rabbits were removed from the box to a large cage after the temperature had been constant for some time. The release was invariably followed by an abrupt and marked rise as shown in curve *C*.

Curve *B* gives a specimen of the temperature variations of rabbits kept in the manner described above, in a box admitting of no muscular movement. The small range of variation and the lack of fluctuation are very evident. Thirty-one observations of daily variations were made on 19 rabbits with nearly identical results. A comparison of this curve with curve *A* of a rabbit at large in a cage shows in the former a nearly

uniform and non-fluctuating norm, departure from which could with a fair degree of certainty be ascribed to any known experimental cause; in the latter, large uncertain deviations of doubtful origin. It is true that many rabbits in the cages maintained a fairly constant temperature, as constant in fact as many in the boxes; but this condition was unpredictable. Those in the quiet of the box, though often showing some deviation, never in any instance had marked extremes.

I have used this method far more than two years on some 150 rabbits in my studies on the regulation of body temperature and have found that marked variations occur only after experimental procedure.

SUMMARY

Rabbits kept in small boxes with a Zwarmak head holder do not indulge in muscular exercise and therefore have a nearly constant body temperature. This makes it possible to study small experimental temperature changes with a degree of exactness not possible under the usual wide normal range of variability of rabbits not in a similarly controlled experimental environment.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

V. The Temperature Effects of Different Concentrations of Sodium Chloride Solution Intravenously Administered

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Received for publication March 8, 1921

I have shown in a former paper (1) that an increase in intracranial pressure is followed by a rise in body temperature in rabbits. The increase in pressure was brought about by mechanical means, but might be produced in various ways. The method of injecting salt solution intravenously to increase or decrease the cerebro-spinal pressure was suggested by the work of Weed and McKibben (2) in which it was shown that hypotonic solutions increase and hypertonic (saturated) solutions decrease the cerebro-spinal pressure in cats.

It seemed of interest to further test the possibility of a correlation between cerebro-spinal pressure and body temperature by observing the temperature effects of similar salt injections and their relation to the pressure effects noted by these workers. It was expected that the rise in the cerebro-spinal pressure produced in this manner would be paralleled by a rise in body temperature similar to that following mechanically increased pressure. The fall in cerebro-spinal pressure might correspondingly be accompanied by a fall in body temperature.

Five to 10 cc. of distilled water were used as the hypotonic solution and the same quantity or less of 6 m (saturated) NaCl as the hypertonic solution. Injections were made in the marginal vein of the ears of rabbits and caused no apparent discomfort or disturbance. Temperature readings were immediately begun and continued at 2 to 15 minute intervals until the maximum change had been reached and a return to normal had begun.

Since normal fluctuations are slight when the rabbit is fastened in the operating box (3) and as there were no variations due to anesthesia or operative procedures (4), a steady rise or fall in temperature followed

by a return to normal was considered as due to the effects of the salt solution injected.

Distilled water, curve *A*, caused a gradual average rise of 1°C. with a gradual return to normal. 6 m NaCl, curve *F*, which is practically a saturated solution, caused an abrupt average fall of 0.6°C. in 5 to 20 minutes followed by a rise to or above normal in 25 to 30 minutes. The marked initial fall in temperature is the characteristic effect of the 6 m NaCl. It occurred in 24 of the 30 cases of injection. The six rabbits in which a rise after injection of 6 m NaCl was observed instead of a fall had more or less severe convulsions following the administration. This is apt to happen if the injection is too rapid or the dose too large. The excessive muscular movements are no doubt responsible for the rise in temperature in these exceptional cases.

The increase in body temperature due to distilled water, hypotonic solution, and the fall due to 6 m NaCl, hypertonic solution, correspond roughly to the rise and fall in cerebro-spinal pressure following injection of the same concentrations as reported by Weed and McKibben (2).

Table of temperature changes following intravenous injections of NaCl

	NUMBER OF RABBITS USED	NUMBER OF INJECTIONS GIVEN	SUBSTANCE INJECTED	AMOUNT INJECTED	AVERAGE CHANGE IN TEMPERATURE	AVERAGE TIME PRECEDING MAXIMUM CHANGE
				cc.	*°C.*	*minutes*
1	2	3	Distilled water	5–10	+1.0	120
2	3	10	M/6 NaCl	5–10	+1.25	90
3	3	10	M/1 NaCl	5	+1.4	60
4	2	7	2 M NaCl	5	+1.5	60
5	3	8	4 M NaCl	5	+1.5	60
6	15	30	6 M NaCl	4–10	−0.6	20
					+0.4	90

In addition to the temperature effects of hypotonic and very concentrated hypertonic NaCl solutions, those of isotonic and more dilute hypertonic solutions were observed; m/6 NaCl, curve *B*, caused a rise similar to that of distilled water; m/1, 2 m and 4 m NaCl, curves *C*, *D*, *E*, although hypertonic, were followed by a rise which came on more rapidly and was of greater magnitude than that of distilled water or m/6 NaCl. There was an average rather abrupt rise of 1°C. with m/1, a more abrupt rise of 1.5°C. with 2 m and 4 m NaCl. In a few cases the rise after 4 m NaCl was preceded by a slight initial fall. It will be seen that the point of reversal between a temperature increasing and a

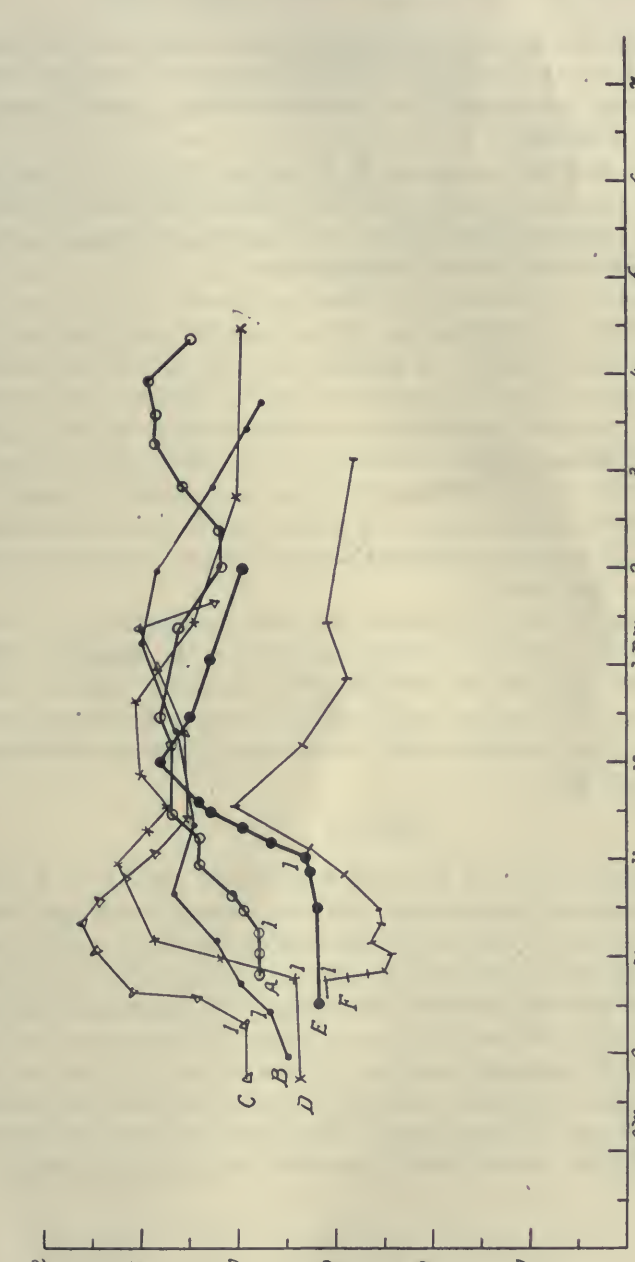

Fig. 1. *A.*—Temperature curve following injection of distilled water. *B.*—Temperature curve following injection of $\frac{M}{6}$ NaCl. *C.*—Temperature curve following injection of $\frac{M}{4}$ NaCl. *D.*—Temperature curve following injection of 2M NaCl. *E.*—Temperature curve following injection of 4M NaCl. *F.*—Temperature curve following injection of 6M NaCl.

temperature decreasing effect—the same quantity being used with each —is between 4 m and 6 m NaCl not between hypotonic and hypertonic solutions as would be expected from the effects of the same solutions on cerebro-spinal pressure.

The discussion of the results will be given in a later communication in connection with further data which are being obtained on the actual cerebro-spinal pressure, blood volume and vasomotor changes accompanying the rise and fall in body temperature.

It is of interest to note that while these experiments were in progress the work of Weed and McKibben (2) was repeated and their general results confirmed by Foley and Putnam (5), who also introduced the salt into the gastro-intestinal tract with similar results. The clinical application of the same principle was made by Cushing (6) who found that the brain volume could be altered by the administration of hypertonic salt solution per os and that the brain is peculiarly sensitive to movements of water in the tissues. Also Sach and Belcher (7) are using this method to decrease intracranial pressure before opening the dura in brain tumor operations.

Thus, it seems to be an established fact that the movement of water in the tissues due to changed osmotic conditions in the blood does alter the bulk of the brain and the cerebro-spinal pressure. And the evidence presented in this paper would seem to indicate that there is also a correlation between changes in the cerebro-spinal pressure and changes in body temperature.

SUMMARY

Distilled water, m/6, m/1, 2 m and 4 m sodium chloride in 5 to 10 cc. doses intravenously administered cause an average rise in body temperature of 1 to 1.5°C.; 6 m sodium chloride, on the other hand, causes an initial fall of 0.6°C. followed by a rise to or slightly above normal. The rise in temperature following the injection of hypotonic solutions corresponds roughly to the rise in cerebro-spinal pressure; and the fall following 6 m (hypertonic) sodium chloride to the fall in cerebro-spinal pressure after injections of hypotonic and hypertonic solutions as reported by Weed and McKibben. This seems to further indicate a correlation between cerebro-spinal pressure and body temperature.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 56 JULY 1, 1921 No. 3

THE GASTRIC JUICE IN PANCREATIC DIABETES

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Received for publication March 8, 1921

Rabens (10) in his studies on blood transfusion from normal into diabetic dogs, has found, incidentally, that on the same diet the diabetic dogs eliminated in the urine only from about one-seventh to one-tenth of the chlorides found in the urine of normal dogs.

Verhaegen (13) reports three diabetic patients, two examined by himself and one by Kultz, whose gastric juice did not contain any hydrochloric acid.

Great hunger and appetite and increased capacity to digest meat have been observed in dogs with pancreatic diabetes.

To throw more light upon the above-mentioned observations, and to search for a possible relation between the secretion of the pancreas and that of the stomach, the study of the gastric juice in experimental pancreatic diabetes was undertaken.

EXPERIMENTAL METHODS

Operation: The abdominal incision for the Pavlov stomach pouch was made to the left of the linea alba and the opening was brought out 2 cm. to the left of the umbilicus. The pancreas was removed at one step, the abdominal incision being made to the right side of the linea alba.

The gastric juice was collected by inserting a rubber tube, perforated at several places, into the pouch. A glass tube was inserted into the external orifice of the rubber tube so as to insure the drainage of the gastric juice to the outside, some distance from the abdominal wall. A small bottle, into which the glass

tube drained the gastric juice, was fastened to the trunk of the dog by means of straps.

In order to avoid all inhibitory influences, the animals were never kept in frames. In the active secretion period they were constantly watched to prevent any attempt of an animal to turn on its back and thus spill the gastric secretion.

The acidity of the gastric juice was determined by titrating with $\frac{1}{10}$ N NaOH, using dimethyl-amino-azobenzol and phenolphthalein as indicators.

The chlorides were determined according to the Volhard-Harvey method. However, instead of 2 cc. $\frac{1}{2}$ N ammonium thiocyanate being equal to 1 cc. of N AgCl, 4 cc. of $\frac{1}{4}$ ammonium thiocyanate solution were made equal to 1 cc. N AgCl, thus insuring more accurate results. In titrating according to the Volhard-Harvey method the first change of color throughout the mixture must be taken as an end point. If the solution which is being titrated is left to stand for a very short period the thiocyanate will have a solvent effect upon the silver chloride, and the light brown color of the end point will disappear, and thus further titration will give inaccurate results.

The pepsin activity of the gastric juice was determined by the Mett's egg albumin tube method, modified by Carlson, and calculated according to the Shutz law.

RESULTS

In order to demonstrate the character of the gastric juice before and after total pancreatectomy with respect to the secretion curve and the chemistry of the gastric juice, a few tables are inserted.

TABLE 1

The average hourly secretion of gastric juice

HOURS	DOG 7		DOG 9	
	Normal*	Diabetic†	Normal‡	Diabetic§
	Average diet 292 grams cooked meat	Average diet 260 grams cooked meat	Average diet 270 grams raw meat	Average diet 125 grams raw meat
1	11.8	3.7	9.0	1.8
2	8.8	7.4	8.1	3.5
3	7.4	10.9	6.9	5.8
4		11.8	6.7	5.5
5		11.1	6.0	8.1
6		10.5	6.3	6.9
7		4.3	6.6	3.4
8		3.2	4.4	4.7
9		5.3	4.0	5.6
10		5.4		4.9
11				6.8
12				1.0

* Average of 12 experiments.
† Average of 7 experiments.
‡ Average of 4 experiments.
§ Average of 5 experiments.

The secretion curve. From table 1 it is evident that on a diet of meat the normal animal secretes the highest quantity of gastric juice in the first 2 hours. The curve of secretion then gradually declines, but the secretion period lasts from 4 to 8 hours. This curve, as obtained by us on normal dogs having a meat diet, is evidently the same as the curve obtained by Pavlov under similar conditions. Dogs 7 and 9, before total pancreatectomy, yield the highest quantity of gastric juice in the first hour and the yield then declines with some fluctuations, but the active secretion period lasts over 9 hours.

After total pancreatectomy dog 7 secreted in the first 2 hours less than he did in the normal condition; the secretion rising in the 3rd, 4th, 5th and 6th hours; the greatest being obtained in the 4th hour, followed by decrease in the 7th hour and another increase in the following hours. The secretion period lasted over 11 hours. Dog 9, after total pancreatectomy, has secreted in the first hour less than in any other single hour of the active secretion period. The curve rose in the 3rd, 4th, 5th and 6th hours, the peak was reached in the 5th hour; followed by a sudden drop in the 7th hour and another rise in the following hours. The active secretion period lasted more than 12 hours. Thus the secretion curves in both dogs after total pancreatectomy are apparently alike, and quite different from the normal secretion curve.

The lengthening of the secretion curve in diabetic dogs is evidently due to the lasting spasm of the pylorus on the duodenal side. Pavlov (9) observed that the passage of acid solution out of the stomach is remarkably slower in the case of dogs with pancreatic fistula. Tobler (12) and Lang (7) have shown that acid protein on the duodenal side checks gastric evacuation. Hedblom's (6) experiments show that potato with 0.25 per cent acid passes out of the stomach more slowly than normal potato, and faster than potato of 1 per cent acidity. Later, Boldyreff (1) showed that fluid of 0.5 per cent acidity passes out quickly from the stomach into the duodenum and the acidity of the fluid remaining in the stomach is reduced to about 0.15 per cent. When the pancreatic ducts are ligated, the acid fluid remains in the stomach for a longer period, and the acidity is reduced to only about 0.40 per cent. Cannon's results are to the effect that the acidity on the duodenal side closes the pylorus. These observations lend support to our contention that the lengthening of the secretion curve of gastric juice after total pancreatectomy is due to the absence of the external secretion of the pancreas, which according to Boldyreff is the chief factor in neutralizing the acid contents of the stomach and duodenum. With the absence

of the pancreatic juice, the pyloric sphincter on the duodenal side is thrown into a lasting spasm, retaining the food in the stomach. The longer the food remains in the stomach, the more the secretagogues are formed, and therefore the greater is the secretion of gastric juice. It would seem, therefore, that we have an adaptation by which the food is subjected to pepsin-hydrochloric acid digestion for a longer period than under the normal conditions.

Is there an absence of psychic secretion in pancreatic diabetes? The quantity of gastric juice which a dog yields in the first 2 hours of the active secretion period is equal to one-half of the total amount of that of the whole secretion period (3). The great quantity of secretion obtained in the first 2 hours is explained by the factor of psychic secretion, which is added to the secretion brought about by the action of the secretagogues.

In the diabetic dogs (table 1) the secretion in the first 2 hours is less than that in the normal condition.

In view of the polyphagia present in pancreatic diabetes the question naturally arises: "Is the polyphagia due to hunger or appetite?" According to Luckhart's (8) observations, one of the factors of polyphagia is hunger. The hunger contractions persist after the removal of the pancreas and become more intense with the progress of the disease. As is evident in table 1, concerned with secretion after total pancreatectomy, the first 2 hours of secretion are scanty. The above considerations would suggest that these hunger contractions are not a factor in the production of secretion during the first hours in the normal animal, and that the psychic factor of secretion is absent or impaired after pancreatectomy.

The development of gastritis after total pancreatectomy in dogs with Pavlov stomach pouches. After total pancreatectomy all the dogs developed symptoms of acute gastritis on the 2nd or 3rd day after the operation. They were constantly retching and vomiting. Dog 2 and dog 3 died on the 3rd day after the operation. The post mortem revealed a highly hemorrhagic condition of the stomach and duodenal mucosa. Dogs 7 and 9 also developed symptoms of gastritis. Dog 7 lived 8 days after pancreatectomy and suddenly died. The stomach mucosa showed a highly hemorrhagic condition. Dog 9 was retching and vomiting after the operation for 2 days, but then the symptoms disappeared. This condition of gastritis has not been mentioned, nor apparently ever noticed after total pancreatectomy.

The acidity of the gastric juice after total pancreatectomy is not changed to any considerable degree. Table 2 indicates that after total pancreatectomy the free acidity of the gastric juice in dog 2 was 0.3646 which is about the same as the average acidity of the animal in the normal condition. Dog 3 gave a free acidity of 0.2918 in the single experiment obtained, which is somewhat lower than the average acidity of the same dog in the normal condition. Dog 7 before pancreatectomy gave a free acidity of 0.4224, while after pancreatectomy the average free acidity was brought up to 0.4403. Dog 9 showed a free acidity of 0.4146 before total pancreatectomy and 0.3104 after the pancreas was removed. As indicated by table 2, the percentage of chlorides of the gastric juice before and after pancreatectomy is about the same. The above

TABLE 2

Average acidities. Pepsin concentration and chlorides before and after pancreatectomy

NUMBER OF DOG	FREE ACID		TOTAL ACID		PEPSIN		CHLORIDES	
	Normal	Diabetic	Normal	Diabetic	Normal	Diabetic	Normal	Diabetic
2	0.3630	0.3646	0.4199	0.4010	3.13	6.25	0.50	0.45
3	0.3927	0.2918	0.4320	0.3646	2.31	36.0	0.48	0.46
7*	0.4274	0.4403	0.4585	0.4762	1.51	3.98	0.49	0.49
9†	0.4146	0.3104	0.4527	0.4242	5.55	10.78	0.51	0.46

* Average of twenty-two experiments in the normal dog and thirty-three in the diabetic dog.

† Average of twenty-seven experiments in the normal dog and twenty-two in the diabetic dog.

considerations clearly indicate that there is not any obvious or constant difference between the acidity of the gastric juice in the normal and in the diabetic conditions.

The pepsin concentration of the gastric juice is increased after total pancreatectomy. Table 2 indicates that dog 2 gave an average of 3.13 mm. of digestion in the normal condition; while after pancreatectomy the single experiment gave a peptic concentration of 6.25 mm. Dog 3 gave an average of 2.31 mm. digestion before pancreas was removed, while after pancreatectomy the single experiment shows 36 mm. digestion. Dog 7 gave an average 1.51 mm. of digestion in the normal condition, while after pancreatectomy the pepsin concentration increased to 3.98 mm. Dog 9 gave an average of 5.55 mm. digestion in the normal condition; after pancreatectomy the average pepsin concen-

tration reached 10.78 mm. Thus, there is conclusive evidence that the pepsin concentration of the gastric juice after total pancreatectomy has been increased.

GENERAL CONSIDERATION OF THE ACIDS, CHLORIDES AND PEPSIN OF THE GASTRIC JUICE

The data accumulated in the examination of the gastric juice in the normal animal warrant some remarks in regard to the acidity, chlorides and pepsin concentration.

The free acidity of the gastric juice according to most observers fluctuates between 0.4 per cent and 0.5 per cent. Our data accumulated in the examination of many samples from various dogs, confirm the above

TABLE 3

Showing the relation between rate of secretion and acidity

NUMBER OF DOG	DIET	NUMBER OF EXPERIMENTS	NUMBER OF SAMPLES EXAMINED	AVERAGE QUANTITY PER HOUR	FREE ACIDITY	TOTAL ACIDITY
				cc.		
2	Meat and bread	12	23	2.0	0.2239	0.2792
2	Meat and bread	7	17	3.5	0.3630	0.4199
3	Meat and bread	9	17	2.1	0.1443	0.2974
3	Meat	12	23	4.3	0.3927	0.4320
4	Meat and bread	3	7	1.1	0.0876	0.1699
9	Meat	12	28	9.8	0.4274	0.4585
8	Meat	10	20	19.9	0.4486	0.4822
9	Meat	4	23	8.8	0.4146	0.4527
13	Meat	1	8	8.8	0.4272	0.4905

view. The low acidities obtained in the examination of the gastric juice are evidently due to the slow rate of secretion. If the rate of secretion is low the contact of the acid secretion with the alkaline mucus is more prolonged and the acidity is reduced, due to the neutralizing and diluting factor of the mucus. Table 3 shows that the slow rate of secretion gives low acidity. When the rate of secretion increased due to a change of diet, the acidity of the gastric juice made a striking increase.

The chlorides of the gastric juice have been examined in a number of samples collected from different dogs. Before examination the juice was put through cotton or gauze eliminating the particles of mucus. Tables 2, 4 and 5 indicate a striking uniformity of the chlorides. The chlorides are generally in direct proportion to the acids

when the acids are high. When the acids are low they have a relatively high chloride content. If we assume that the mucus which is mixed with the gastric juice is low in chlorides, the corresponding high chlorides of the gastric juice with low acidities are probably due to the strongly neutralizing effect of the mucus. Our observation on the uniformity of chlorides in the gastric juice confirms the work of Roseman (11).

TABLE 4

Showing relation of pepsin concentration to acidity and chlorides

NUMBER OF DOG	NUMBER OF SAMPLES EXAMINED	FREE ACIDITY	TOTAL ACIDITY	PEPSIN	CHLORIDES
7	7	0.4688	0.4981	3.22	0.52
7	7	0.4591	0.4866	3.00	0.51
7	5	0.4503	0.4849	4.00	0.48
7	9	0.4433	0.4527	4.73	0.48
7	10	0.3728	0.4284	4.97	0.47
9	5	0.4388	0.4594	4.63	0.52
9	4	0.4078	0.4557	5.09	0.51
9	7	0.3948	0.4388	5.91	0.51
9	12	0.1854	20.704	18.7	0.43
9	1	0.0456	0.0912	31.36	0.46

TABLE 5

Continuous secretion of dog 9

HOURS	QUANTITY	FREE ACID	TOTAL ACID	PEPSIN	CHLORIDES
Normal					
	cc.				
9 p.m.–9 am.	20.0	0.0456	0.0912	31.36	0.46
9 a.m.–1 p.m.	5.6	0	0.0549		0.46
Pancreas removed					
8:15 p.m.– 6:30 a.m.	12.0	0.1732	0.2644	16.00	
7:40 p.m.– 7:30 a.m.	18.0	0.1732	0.2827	7.29	
3:30 p.m.– 8:00 a.m.	15.0	0.1732	0.2644		0.39
9:30 p.m.– 5:00 a.m.	12.0	0.2827	0.3281	16.00	0.39
10:10 p.m.– 8:45 a.m.	7.0	0.1185	0.2371	21.16	0.42
10:00 p.m.– 9:00 a.m.	9.0	0.1459	0.2188	34.81	0.46
	9.0	0.2280	0.3646	16.00	0.46
10:00 a.m.– 1:35 a.m.	3.0	0.2188	0.2918	13.69	
1:35 p.m.– 6:35 p.m.	5.0	0.1641	0.2280	22.09	
6:30 p.m.–11:00 p.m.	6.0	0.1641	0.1732	15.21	
11:00 p.m.– 9:00 a.m.	8.0	0.1276	0.2097	16.81	

The continuous secretion is high in pepsin but low in acid. Table 5 gives the continuous secretion of dog 9 before and after pancreatectomy. The gastric juice is low in acid, but high in pepsin. Our findings are in accord with Carlson's observations on continuous secretion in man.

The gastric juice collected during the active secretion period does not show any direct proportion between the acidity and pepsin concentration as noticed by Edkins and Brinkman (2). On the contrary, there is some evidence that gastric juice low in acidity is higher in pepsin as is indicated in tables 4 and 5.

The flocculent, ropy mass, separated from the gastric juice is high in pepsin, but very low in acid. Samples of gastric juice, containing mucus were allowed to stand for a period of from 24 to 48 hours. At the bottom of the test tube a flocculent, ropy mass is then generally found. The examination of this ropy mass proved to be two to four times as high in pepsin as the gastric juice of high acidity which was collected during the active secretion period.

CONCLUSIONS

The secretion of the gastric juice after total pancreatectomy in dogs with Pavlov stomach pouches, is different from the one obtained under normal conditions:

1. The quantity of the first 2 hours is less than that in the normal dogs, indicating the possible disturbance of the mechanism concerned in psychic secretion; the quantity secreted in the 3d, 4th, 5th, and 6th hours of the active secretion period is greater than in the normal curve; the active secretion period is prolonged, lasting twelve or more hours.
2. The total quantity of gastric juice secreted on the same diet has been more than doubled;
3. The pepsin concentration of the gastric juice is greatly increased;
4. The acidity of the gastric juice in pancreatic diabetes does not essentially differ from the normal.

The hyper-secretion of gastric juice, the increase in pepsin concentration, and the longer retention of the food in the stomach, point to an adaptation to prolong and augment the pepsin-hydrochloric acid digestion after total pancreatectomy.

A typical severe gastritis, with constant retching, and vomiting has been observed after total pancreatectomy on dogs with Pavlov stomach pouches.

The free acidity of the normal gastric juice during the active secretion period does not fluctuate much, being between 0.3630 and 0.4146, and the total acidity between 0.4199 and 0.4527. This confirms the work of Pavlov, Carlson, Boldyreff and others.

The chlorides of the gastric juice are uniform, thus confirming the work of Roseman.

Continuous secretion is low in acid but high in pepsin, which confirms the work of Carlson on human gastric juice.

The thick flocculent mucus separated from the gastric juice is high in pepsin, but very low in acid.

I wish to express my thanks to Dr. J. J. Moorhead for his generous help in some of the operations. I am deeply indebted to Doctor Carlson who suggested this problem.

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STUDIES OF THE THYROID APPARATUS

II. The Changes in the Amount of Intestine-Contracting Substances of the Thyroid of the Albino Rat According to Age

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Received for publication March 10, 1921

It is a well-known fact that aqueous, saline and other extracts of the thyroid gland of various animals cause contraction of the isolated intestinal segment from laboratory animals. Nevertheless since this property is exhibited by extracts of other tissues, such as thymus, spleen, brain, kidney, liver, pancreas, parathyroid, intestinal mucosa and musculature, heart, striated muscle, etc., as Wieland (1), Köhler (2), Fawcett and his collaborators (3), Stern and Rothlin (4), Collip (5) and others have shown, the production of the substances causing the contraction can not be considered as a specific function of any particular tissue. Indeed it is becoming increasingly evident that there are substances elaborated by, or contained in, many tissues of the body which exhibit a common type of effect on the blood pressure, gastric secretion, heart-beat and other physiological processes, and that this general type of reaction must be distinguished from the specific action of extracts of special glands, as Clark (6) has recently emphasized. Attention should be called to the fact that this similarity of type of reaction production by extracts from such obviously functionally diverse organs indicates an underlying fundamental similarity in metabolic procedure of the various tissues and organs of the body as a whole, upon which has been superimposed, through some process of evolution or development, the more prominent physiological function commonly attributed to the particular organ in question.

It is not the purpose of this report to consider the probable nature of the active substances in the extracts, since we have but little if any actual information with regard to this point. Rather our purpose is to present as briefly as possible the changes in the amounts of the

intestine-contracting substances of the thyroid of the albino rat according to age, as measured by the reaction induced in isolated duodenal segments of the rat by extracts of this gland.

The isolated duodenal segment of the adult, unexcited, male albino rat was chosen as the test organ for this investigation because of its qualitative and quantitative reliability for comparative studies of this

TABLE 1

The age and sex distribution of the animals the thyroid extracts of which were tested, together with the averages of the intestine-contracting power of the extracts according to the individual age groups

MALES				FEMALES			
(1) Age	(2) Number of tests	(3) Number of rats	(4) Contraction in terms of M/10 Na_2CO_3	(5) Age	(6) Number of tests	(7) Number of rats	(8) Contraction in terms of M/10 Na_2CO_3
days			*per cent*	*days*			*per cent*
New-born	3	15	73.8	New-born	4	31	86.5
10–11	3	9	41.0	10–11	2	10	64.0
15	3	6	47.2	14–15	2	6	92.9
20	3	6	56.8	21–22	2	4	72.2
25	3	6	43.4	25	2	3	111.6
30	3	6	83.9	30	2	3	45.0
40	3	3	41.3	39	2	2	52.9
50	3	3	63.0	50–52	3	3	83.3
60	3	3	70.5	54–55	3	3	63.9
70	4	4	75.9	60	2	2	86.1
79–80	3	3	167.5	70	2	2	72.1
90	2	2	119.8	75	2	2	72.1
100	1	1	121.4	81–83	3	3	105.0
125	3	3	138.6	90	3	3	99.5
150	3	3	228.8	100	3	3	101.7
200	3	3	101.7	125	1	1	95.0
460	1	1	38.1	150	4	4	164.6
500	2	2	59.2	300	4	4	38.6
520	1	1	68.4				

type, as was shown by Hatai and Hammett (7) and Hammett (8). The method of standardization of such a segment has been described in an earlier paper (8) and needs no elaboration.

The extracts, the relative strength of which was tested, were prepared from the thyroid glands of albino rats of ages ranging from newborn to 500 days. All the animals used were healthy and from the standard stock of The Wistar Institute colony. Values were obtained

for both males and females. The thyroid was removed from the animal, under deep ether anesthesia, was weighed and ground in a small mortar with fifty times its weight of fine dry sand to which was added during the grinding that amount of Tyrode's solution (without sugar) which would suffice to make the concentration of the thyroid in the final solution equivalent to 0.25 per cent. Thus in all cases equal

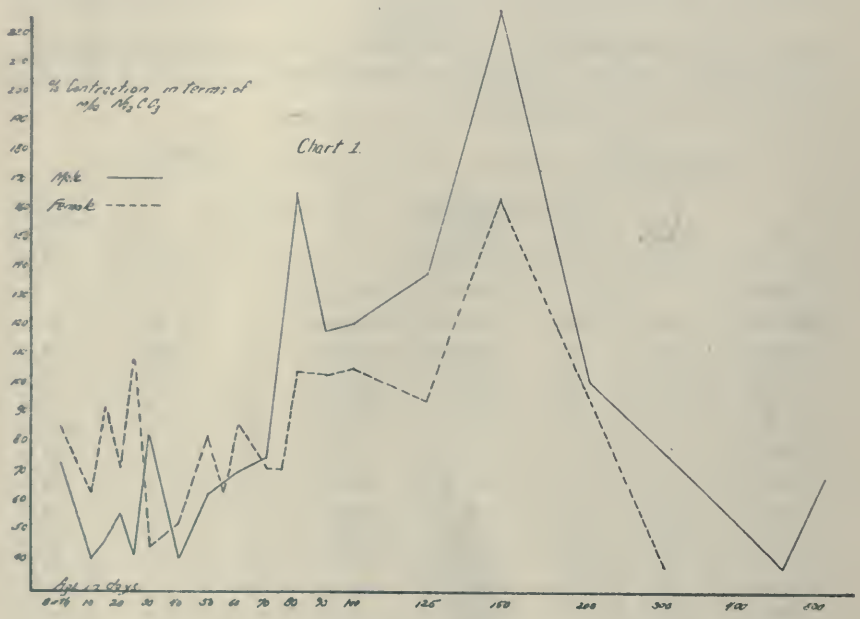

Chart showing the changes in the intestine-contracting power of equivalent amounts of thyroid extracts from male and female albino rats according to age.

amounts of extract from each and all glands were representative of equal unit weights of fresh thyroid tissue. The segments were standardized with 0.25 cc. of M/10 Na_2CO_3 and the shortening induced by 1 cc. of the thyroid extract was compared therewith and expressed as the per cent contraction in terms of M/10 Na_2CO_3.

In all a total of 93 tests was made on 163 animals. The details of the age and sex distribution are given in table 1. The figures in columns 4 and 8 are the average values obtained in the individual age groups studied. These values have been plotted in chart 1. It should be obvious that the size of the thyroid in the very young rats necessitated the use of glands from several animals for each test of the earlier ages.

An inspection of the chart reveals several interesting features. Foremost among these, for purposes of validating the investigation as a whole, is the quite general coincidence of the graphs for the two sexes with regard to the type of change occurring with age. This demonstrates that apparently no marked qualitative sex differences occur, which indicates that the function of these intestine-contracting substances, if they have a function, subserves the same ends in both the male and the female albino. The fact that the course of the curve for the females, while similar to that of the males, precedes by a few days the changes occurring in the graph of the males falls in rather nicely with the observations reported by Donaldson (9) that certain other physiological changes in the female albino rat, such as the opening of the eyes and the onset of puberty, usually occur slightly before they take place in the males, and supports the idea that we are here dealing with an expression, indirect it is true, of a fundamental uniform difference in the development of the two sexes.

If we assume that the variations in the intestine-contracting ability of the thyroid extracts tested are due to variations in the amount of stimulating substances produced per unit weight of thyroid gland, it is at once evident that there are four periods during the active growth of the albino rat when the activity of the thyroid in producing this stimulating complex is noticeably increased: *1*, at birth; *2*, at the period of weaning; *3*, at puberty; and *4*, at the termination of rapid growth, or 150 days. Following each of these periods of higher content of stimulating substances or greater activity in their production, there occurs a marked decrease in the content of the extracts as tested by their action on the isolated intestinal segment. There is the post-natal drop; the post-weaning drop, which is followed by a gradual increase in activity up to the time of the jump to the pubertal high-point; the post-pubertal decrease or, in the females, the post-pubertal plateau; and finally the big decline in activity occurring after the animals have reached full maturity and which is apparently under normal conditions more or less the expression of the activity of the extracts of the thyroid of adult animals. The general trend through all the ups and downs up to the time of this regression is toward an increase in activity. The jog at the time the eyes are opening (about 15 days) may be significant.

The jogs in the curves at weaning and at puberty are similar in their incidence to those observed by Hatai (10) in his study of the refractive index of the blood of the albino rat and when correlated with data that is now accumulating relative to the fatalities following thyreopara-

thyroidectomy at different ages, point strongly to the probability that these two periods, e.g., weaning and puberty, are critical points in development, and points deserving of special investigation. Whether or not they are periods at which the thyroid plays a particular and significant rôle is yet to be determined.

Other studies of evidences of changes in thyroid activity with age have been made. Fenger (11) found that the iodine content of this tissue varied with age, and since he was of the opinion that "the amount of iodine in the thyroid is an indication of the relative activity of the gland" he concluded from his analyses that "there is evidently a gradual rise in activity of the gland in the fetus and this activity is increased shortly after birth, reaching its maximum in the young growing animal." "The iodine content of full-grown animals is very low." This opinion and the findings on which it is based parallel the curves we present. Fenger's studies were made on thyroid glands from beef, sheep and hogs. Moroever Robertson (12) in a brief comparison of the relation of the thyroid weight to body weight of the white mouse, presents figures which show the maximum ratio to exist in animals 70 days of age, while in the adult animals of 210 days the ratio has fallen to that present at 35 days. Since these three separate and distinct lines of investigation give evidence of the same type and direction, it is relatively safe to conclude that a phase of the activity of the thyroid gland, aside from all other factors, changes with the progress of development of the organism.

It is not our intention to give the impression that the tests of the thyroid extracts as carried out in this investigation are to be considered as tests of the specific functional activity of the gland, or as expressing variations in the production of its particular internal secretion which Kendall (13) has isolated and named "thyroxin." In fact the evidence at present available justifies only the opinion that the variations observed are but the particular expressions of general changes in the organism as a whole, in which the thyroid gland, as part of that organism, participates. Nevertheless the possibility must not be overlooked that the thyroid may take a directing or regulating part in these changes and that the variations in its content or production of the intestine-contracting substances as products of its activity may be rough indices of variations in its specific functional activity.

SUMMARY

When the effects on the standardized intestinal segment of equivalent concentrations of extracts of thyroid glands from albino rats of different ages are compared, it is found that certain differences in strength are present according to the age of the animal from which the gland was removed.

Apparently there occurs a heightened content or production of the intestine-contracting substances in the thyroid at birth, at weaning, at puberty, and at the time rapid growth is about completed. Each of these points of increased activity is followed by a period in which the extracts are less active in stimulating the intestinal segment, though the general trend is toward an increase until full maturity, when the activity falls to a fairly uniform low level. This holds for both sexes.

A correlation of these variations with data from other sources leads to the opinion that they are the particular expressions of general changes in the organism in which the thyroid participates.

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STUDIES OF THE THYROID APPARATUS

III. The Action of Thyroxin on the Isolated Intestinal Segment

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Received for publication March 10, 1921

In the preceding report it has been shown that extracts of the thyroid gland of albino rats of both sexes at different ages exert a characteristic contracting effect upon the isolated duodenal segment of the same animal.

Since Kendall (1) has shown that the crystalline substance, thyroxin, which he isolated from the thyroid, is an active principle of that gland, in that when it is administered in conditions of hypothyroidism its effect is similar to that of whole gland substance, it seemed worth while to determine whether or not this compound is an active constituent of the thyroid extracts causing the contraction of the isolated intestinal segment. Although the data from other sources indicate that this intestine-contracting ability of the thyroid extracts is not specific but is exhibited by extracts from many other tissues of the body, and although it might appear as if the testing of the possibility of such an effect being produced by thyroxin is superfluous on the basis of the apparent inactivation of the compound when put in alkaline solution, yet since such assumptions of the activity or non-activity of any substance are scientifically dangerous, it was considered that the point should be settled with regard to thyroxin for definiteness if for nothing else.

Since thyroxin is precipitated as the mono-sodium salt from solution in sodium carbonate, if allowed to stand in contact with the air,[1] while it is readily soluble in sodium hydroxide, this latter compound was used in fifteenth molecular concentration, both as the solvent for thyroxin and as the standardizing reagent, instead of the tenth molecular sodium carbonate previously described (2).

One milligram of pure crystalline thyroxin was dissolved in 10 cc. of M/15 NaOH giving a concentration of 0.025 mgm. thyroxin in the 0.25 cc. of solution used for testing and also for standardization. Consecu-

[1] Personal communication from Dr. E. C. Kendall.

tive dilutions with M/15 NaOH were then made which yielded, 5×10^{-3}; 1×10^{-3}; 2×10^{-4}; 4×10^{-5}; 8×10^{-6}; 1.6×10^{-6}; 3.2×10^{-7} and 6.4×10^{-8} mgm. of thyroxin respectively in each 0.25 cc. of solution. A simple calculation will show that the neutralization effect of the thyroxin on the sodium hydroxide, even in the highest concentration, is less than 0.3 per cent, a value extremely small when compared with the limits of accuracy of the method of testing, and which is obviously increasingly diminished with the consecutive dilutions. This wide range of thyroxin concentration was studied because frequently differences in type of physiological reaction to other organic products are observed when

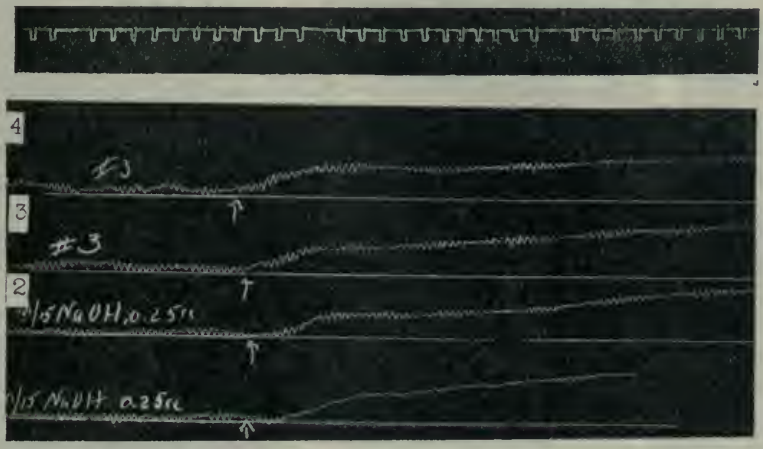

Fig. 1. The effect of 1×10^{-3} mgm. thyroxin on the isolated duodenal segment. The first two tracings are the responses to 0.25 cc. M/15 NaOH as standard; the second two show the response to 0.25 cc. M/15 NaOH plus thyroxin.

different doses are employed. The segments were standardized as described in an earlier report (2), using 0.25 cc. of M/15 NaOH instead of M/10 Na_2CO_3. When the measure of the response of the segment to this amount of NaOH had been obtained the same amount of contracting reagent containing thyroxin of a given concentration was added and its effect compared with the result obtained in standardization.

In no case was there any evidence obtained that thyroxin causes of itself a shortening of the intestinal segment, since in no case did the NaOH solution of thyroxin cause an increase in contraction over that produced by the NaOH itself. The general type of reaction is shown in figure 1.

However, the solutions where thyroxin was present in concentrations of 3.2×10^{-7} and 6.4×10^{-8}, particularly the former, seemed to exert a depressant or retarding effect on the contraction, as is shown in figure 2.

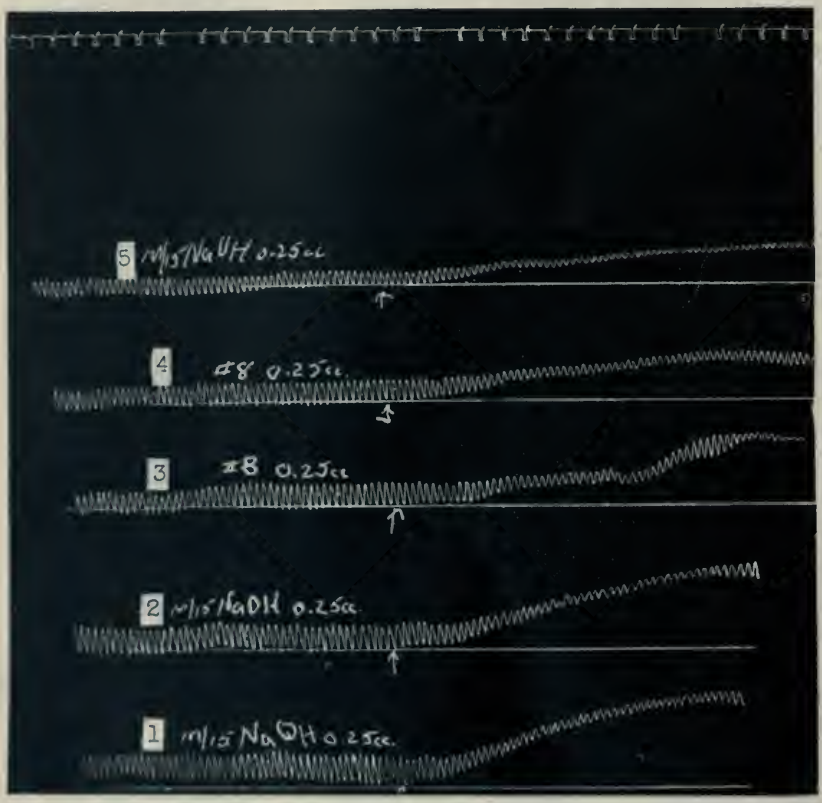

Fig. 2. The effect of 3.2×10^{-7} mgm. thyroxin on the isolated duodenal segment. The first two tracings are the responses to 0.25 cc. M/15 NaOH as standard; the second two show the response to 0.25 cc. M/15 NaOH plus thyroxin; while the fifth tracing is that obtained with 0.25 cc. M/15 NaOH alone.

This amount of thyroxin represents but an extremely small proportion of the iodine present either in normal blood, as determined by Kendall (3), or in the thyroid gland, as reported by Fenger (4), and suggests that the larger proportion of the iodine in the blood or the thyroid gland is not in combination as thyroxin, if this reaction is an expression

of a physiological activity of the compound on the tissue used for testing.

The results do show, however, that thyroxin is not an active constituent of the thyroid extracts, as described in the preceding paper, in causing the characteristic effect on the isolated intestinal segment.

SUMMARY AND CONCLUSION

Solutions of thyroxin in fifteenth molecular sodium hydroxide, in concentrations ranging from 5×10^{-3} to 6.4×10^{-8} do not cause any increased contraction of the isolated intestinal segment over that produced by equivalent amounts of the sodium hydroxide solution used as the standard. At concentrations of 3.2×10^{-7} and 6.4×10^{-8} thyroxin in sodium hydroxide solution apparently tends to exert a repressant effect on the contraction produced by the hydroxide. These are the only concentrations tested at which any physiological effect at all was observed. It is evident from these results that thyroxin is not the constituent of the thyroid extracts which causes the contraction of the isolated intestinal segment described in the preceding paper.

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STUDIES IN CARBON MONOXIDE ASPHYXIA

I. The Behavior of the Heart

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Investigations Performed for the United States Bureau of Mines, in the Laboratory of Applied Physiology, Yale University, New Haven[1]

Received for publication March 15, 1921

Acute oxygen deficiency produces a functional impairment of auriculoventricular conduction. This has been demonstrated by Sherrington, Lewis and Matthison and others (1), (2), (3), (4). The present investigation was undertaken to determine whether the same depression of conduction occurs in animals during carbon monoxide asphyxia; and further, whether this gas exerts a direct toxic action upon the heart, independent of the anoxemia.

It is highly probable that the toxicity of carbon monoxide is wholly dependent upon its union with hemoglobin, and thus upon asphyxia. But even so, the conditions under carbon monoxide differ significantly from those under inhalation of low concentration of oxygen. During carbon monoxide asphyxia the tension of oxygen in the arterial blood remains normal as long as pulmonary ventilation is maintained. It is the quantity which is decreased. On the other hand, during inhalation of low oxygen (as at a great altitude) both the quantity and tension of oxygen in the arterial blood are decreased.

This difference might lead to effects upon the heart varying from those observed in simple asphxyia by the investigators above mentioned.

General procedure. These observations were made upon dogs. A normal electrocardiogram[2] was obtained through leads to the right fore and left hind legs. With the electrodes still connected, the animal was placed in a glass chamber of 300 liters capacity (minus the mass of the animal in liters). An amount of carbon monoxide sufficient to produce the desired concentration was measured in; the accuracy of

[1] Published by permission of the Director of the United States Bureau of Mines.

[2] The apparatus used in this investigation was an Edelmann electrocardiographic outfit presented to the Medical School by Dr. Walter James of New York City.

the concentrations obtained by this method has been proven by previous work (5). With a dog of average size (8 to 10 kilos), the CO_2 in the chamber rose to 0.4 per cent in about 30 minutes and the oxygen fell an amount depending on the respiratory quotient. At the end of each 30 minutes the chamber was opened and rapidly cleared by an electric fan. It was then reclosed and a new charge of gas introduced. The temperature of the chamber rose only a few degrees above that prevailing in the room. Throughout the period of gassing, electrocardiographic tracings were taken at short intervals. In a few experiments respiratory tracings were obtained by means of a pneumograph. To determine the percentage combination of the hemoglobin with carbon monoxide the carmine method of Haldane was used (6). Blood was drawn from the ear.

Rapid fatal asphyxia. In the first seven experiments the asphyxia was carried to a fatal termination in a period of 25 to 40 minutes. The concentration of carbon monoxide ranged from 45 to 70 parts in 10,000 of air.

The general course of the animals' behavior was similar in all of the experiments. The usual excitement stage,—the whole course of events closely resembling ether anesthesia,—was followed by increasing unsteadiness on the feet, depression, and finally unconsciousness. Respiration became noticeably augmented soon after the gassing was started, and developed into dyspnoea which reached its height shortly before unconsciousness developed, and then passed into irregular, or even Cheyne-Stokes breathing. Following this, indications of respiratory failure soon developed. The gasps, which previously had been violent, became slower and feebler until finally respiration ceased entirely. The heart continued to beat for several minutes thereafter.

Until the approach of respiratory failure, the electrocardiographic findings were as follows: The heart rate increased almost from the first. Ventricular extra-systoles occurred in many cases. The T wave became greater in height, as in the observations made by Lewis and Matthison (2) during asphyxia of the spinal cat. In our experiments, however, there was almost uniformly a period of inversion of the T wave, which occurred near the height of the respiratory augmentation. (Note figures 1 and 2.) The heart rate decreased gradually as the volume of pulmonary ventilation lessened in the late stage of the asphyxia.

With the failing respiration the auricular wave, *P*, ceased to register during the expiratory pauses. A peculiar type of respiratory arrhyth-

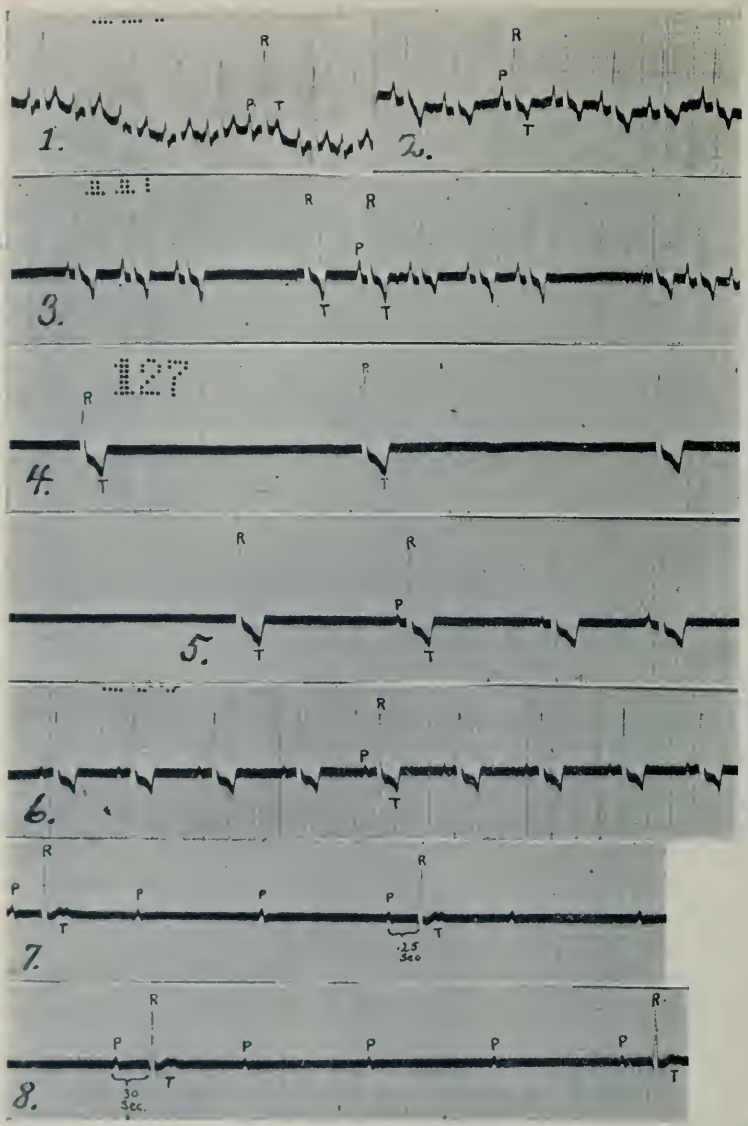


Fig. 1. From dog 1, after 10 minutes exposure to CO. Rate 172 per minute. P–R interval 0.11 second.

Fig. 2. From dog 1, after 18 minutes' exposure to CO. Rate 136. P–R interval 0.11 second. Showing inversion of T wave.

Fig. 3. From dog 1. Type of arrhythmia developed during respiratory embarrassment. No indication of delayed a–v conduction. Cessation of auricular beat during respiratory pause with escape of ventricle. This is seen more clearly in figure 10, which gives respiratory tracing.

Fig. 4. From dog 1. Cessation of auricular beat at time of respiratory failure. Ventricular rate 23 per minute.

Fig. 5. From dog 1. Record continuation of no 4, showing return of auricular wave and increase in cardiac rate.

Fig. 6. From dog 1. Record taken 90 seconds after no. 5. Cardiac rate 91 per minute. P–R interval 0.12 second.

Fig. 7. From dog 1. Development of 3–1 heart block. Auricular rate 59 per minute. P–R interval 0.25 second.

Fig. 8. From dog 1. 4–1 heart block. Auricular rate 59 per minute. P–R interval 0.30 second.

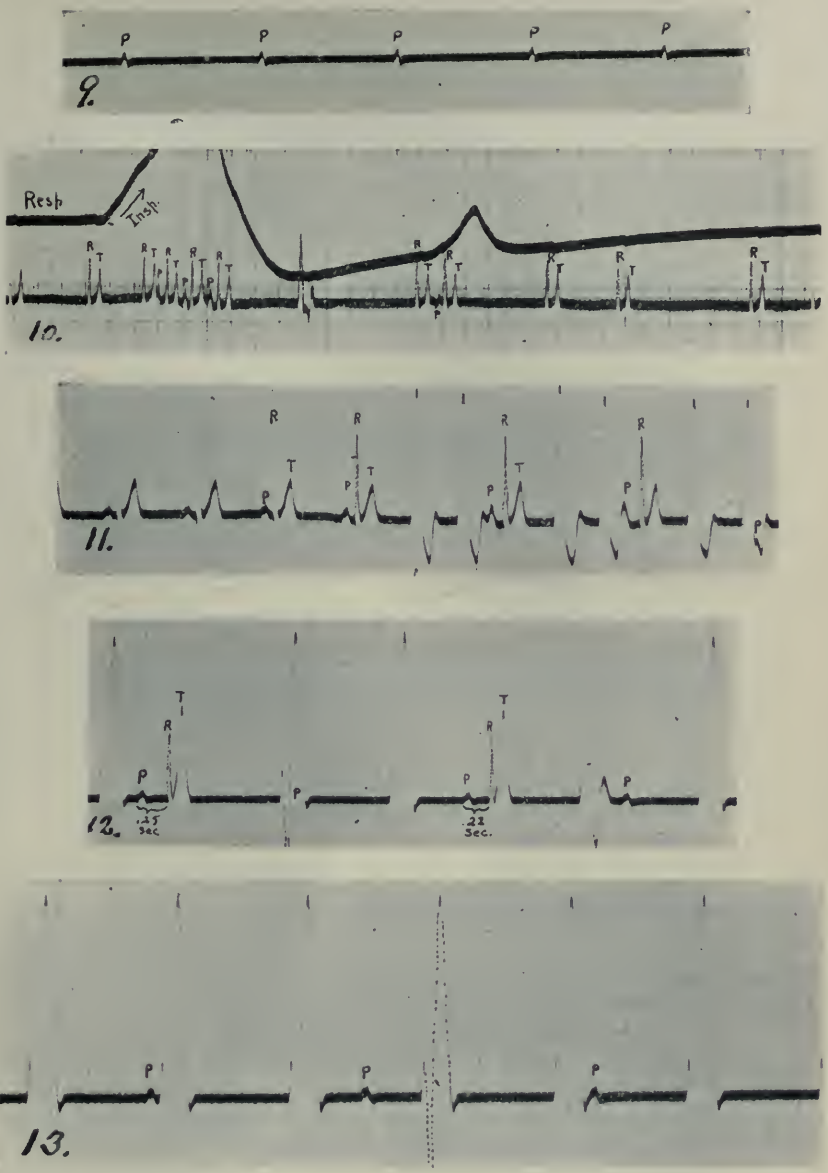


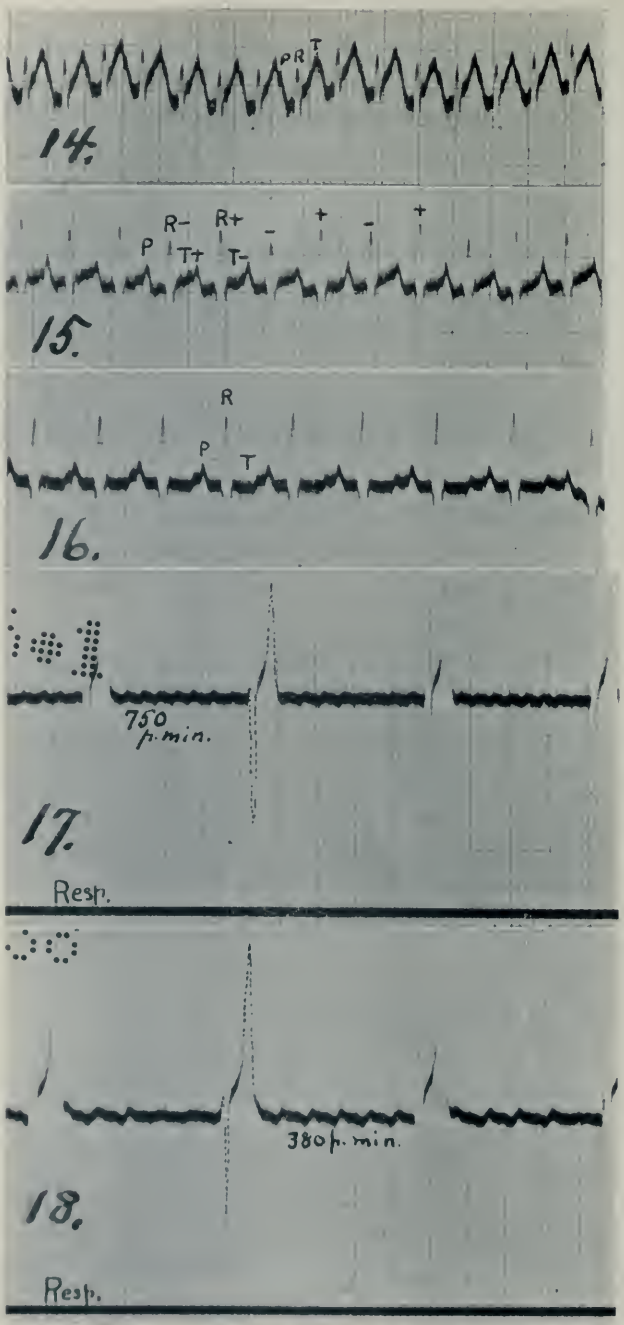
Fig. 9. From dog 1. Complete stoppage of ventricle 5½ minutes after respiratory failure. Auricular rate 59 per minute.

Fig. 10. From dog 6. Type of arrhythmia which develops during respiratory failure. Rapid rate with P wave present, during inspiration Slow ventricular rate, without P wave, during respiratory pause.

Fig. 11. From dog 4. Type of ventricular extra-systoles which develop at the height of the gassing.

Fig. 12. From dog 4; 2–1 heart block, with ventricular extrasystoles. Ventricular rate 48 per minute.

Fig. 13. From dog 4. Complete heart block Auricular rate 38 per minute. Ventricular rate 69 per minute. Origin of ventricular wave other than nodal.



Figs. 14, 15 and 16. Records from dog 9. CO preceded by atropine. Alternation of R and T waves in record 15. This is absent in 14 and 16, which were taken respectively 5 minutes before and after record 15.

Figs. 17 and 18. From dog 6. Taken during complete block. Auricular rate in 17 is 750 per minute; rhythmatic. In 8 the auricular rate is 380 per minute and the waves coarser.

mia developed. With inspiration the heart beat rapidly and the *P* wave was present with no evidence of delayed a-v conduction. During expiration and the following respiratory pause, the P wave was absent and the ventricular rate decreased. Ventricular complexes, apparently of nodal origin, appeared, and continued until the auricle became active with the next inspiration (figs. 3 and 10).

With the complete cessation of respiration the P wave disappeared from the tracings and the nodal ventricular beats assumed a rate of 23 to 40 per minute (fig. 4). By the end of the following minute, evidence of auricular activity had returned with little indication of delay in conduction (fig. 5). With the renewed activity of the auricle, the heart rate increased to 70 or 100 beats per minute (fig. 6). Within the next 2 minutes, delay of a-v conduction became evident in a lengthening of the P-R interval.

From this point two types of activity were presented by the different animals. In one type the impairment in a-v conduction became greater and partial heart block developed, passing through the stages of 1–3 and 1–4 (figs. 7 and 8) to complete block. The ventricular rate gradually became less, until at 4 or 5 minutes after respiratory failure all evidence of ventricular activity had ceased, and the P wave alone registered with a rate ranging from 40 to 60 per minute (fig. 9). This wave continued for 2 or 3 minutes after which the string of the electrocardiograph failed to give evidence of any cardiac activity.

In the second type the delayed conduction developed as in the first, and the various stages of heart block followed. But along with this condition, groups of regularly spaced ventricular extra-systoles occurred at intervals with increasing frequency throughout the records (figs. 11, 12 and 13). The ventricular complexes continued after the P wave had ceased to register. Their rate became slower and their contour more bizarre until they passed finally into a state of ventricular fibrillation.

Protocols 1 and 2 are typical of acute asphyxia.

In one of the seven animals during the period of complete block the auricle presented a rhythmic rate of approximately 750 per minute. The waves gradually slowed during 3 minutes, after which they became irregularly spaced and finally ceased (figs. 17 and 18). The ventricular complexes continued until ventricular fibrillation supervened.

In acute carbon monoxide asphyxia, fatal in periods of less than an hour, the anoxemia produced by the combination of hemoglobin with carbon monoxide is not sufficient in itself to impair a-v conduction. It is only after the great oxygen deprivation caused by failure of respiration that this development occurs.

Protocol 1. Experiment 1. Rapid carbon monoxide asphyxia. Dog 1. Male, 7 kilos. Gassed in 65 parts of carbon monoxide in 10,000 of air

TIME	REMARKS	ELECTROCARDIOGRAPHIC RECORD
min.		
0	Normal record taken. Gas started	Rate 108 per minute. P–R interval 0.12 second. Moderate respiratory arrhythmia.
5	Respiration slightly augmented	Rate 135 per minute. T wave increased in height. P–R interval 0.11 second. No respiratory arrhythmia.
10	Respiration markedly augmented	Rate 172 per minute. P–R interval 0.11 second. (Fig. 1.)
15	Respiration greatly augmented. Animal unable to stand	Rate 210 per minute. P–R interval 0.11 second. T wave decreased in height.
18	Respiration Cheyne-Stokes type	Rate 135 per minute. T wave inverted. P–R interval 0.11 second. (Fig. 2.)
20	Respiration slow, labored and gasping. Animal unconscious	Respiratory arrhythmia. Auricular inhibition during respiratory pause with escape of ventricle. (Fig. 3.)
21	Respiration stopped	Auricular inhibition. Ventricular wave nodal origin 25 per minute. (Fig. 4.)
22		Return of auricular wave. P–R interval 0.12 second. Cardiac rate increasing. (Fig. 5.)
23½		Rate 91 per minute. P–R interval 0.12 second. (Fig. 6.)
25		3:1 heart block. P–R interval 0.25 second. Auricular rate 59 per minute. (Fig. 7.)
26		4:1 heart block. P–R interval 0.30 second. Auricular rate 59 per second. (Fig. 8.)
26½		Ventricle stopped. Auricular rate 59 per minute. (Fig. 9.)
27½		No movement of string.
35	Heart's blood Hb. combined with CO, 86 per cent	

Respiratory failure, and its influence on the heart. The respiratory failure is apparently in the nature of a fatal apnoeavera. The hyperpnoea induced by the deficit of oxygen in the blood results in the elimination of an excessive amount of carbon dioxide. The respiratory quotient during this time is greater than one (as we know from other experiments). The excessive loss of CO_2 has a tendency finally to depress respiration. The respiratory stimulation therefore becomes increasingly dependent upon the deficit of oxygen. A point develops

Protocol 2. Experiment 4. Rapid carbon monoxide asphyxia. Dog 4. Male, 9 kilos. Gassed in 60 parts carbon monoxide in 10,000 of air

TIME	REMARKS	ELECTROCARDIOGRAPHIC RECORD
min.		
0	Normal record taken. Gas started	Rate 96 per minute. P–R interval 0.12 second.
5	Respiration slightly augmented	Rate 126 per minute. P–R interval 0.12 second.
10	Respiration greatly augmented	Rate 170 per minute. Numerous ventricular extrasystoles. P–R interval 0.12 second.
20	Respiration irregular, somewhat labored	Rate 152. P–R interval 0.12 second.
22	Respiration gasping	Rate 125 per minute. P–R interval 0.12 second. Paroxysm of ventricular extra-systole. Decrease in auricular rate. (Fig. 11.)
23	Respiration stopped	No auricular wave. Series of irregular ventricular wave of origin other than nodal.
25		Auricular rate 68 per minute. Regular ventricular response. P–R interval 0.16 second. Numerous interpolated ventricular extra-systoles.
26		2:1 heart block. Auricular rate 50 per minute. P–R interval 0.22–0.25 second. Two or more extra-systoles between each normal ventricular response. (Fig. 12.)
28		Complete block. Auricular rate 38 per minute. Ventricular rate 69 per minute. (Fig. 13.)
29½		Auricular beat absent. Ventricular rate 54 per minute. Complex bizarre.
30		Ventricular complex 32 per minute.
32		Ventricular fibrillation.
40	Heart's blood Hb. combined with CO = 82 per cent	

when either the anoxemia ceases to increase sufficiently rapidly, owing to a flattening of the curve of carbon monoxide absorption, or the respiratory center becomes fatigued or less sensitive to the anoxemic stimulation. The CO_2 of the blood has become abnormally low, and the C_H is consequently decreased also. With the failure of the oxygen deficiency stimulus, respiration ceases, since the normal CO_2 stimulus is now absent.

With the decrease in pulmonary ventilation carbon dioxide reaccumulates to some extent in the blood. The blood alkali at this time has

fallen much below normal (evidence of this will be presented in a later paper) and for this reason, the C_H of the blood rises rapidly with the increase in CO_2. The respiration center fatigued or depressed through the excessive anoxemia to which it has been responding, fails to be stimulated by the rising C_H. As will be seen later it can be driven to further activity by a very considerable increase in the CO_2 of the blood induced through forced inhalation of this gas in proper dilution.

The fatigued cardio-inhibitory center, apparently, maintains its power of functioning after the respiration has failed. Its action is seen in the inhibition of the auricle at this time. This phenomenon, as noted later, is prevented by removing vagal action through injections of atropine prior to gassing. With the further advance of the anoxemia this center also loses its power of functioning and the auricle is freed from the inhibition. The various cardiac activities from this time on are purely those due to the action of oxygen deficiency upon the heart itself.

Influence of atropine upon the cardiac events. As has been suggested in the previous section, atropine abolishes the period of auricular cessation which occurs during the asphyxia at the time of respiratory failure. In four animals, atropine, $\frac{1}{100}$ gr., was administered hypodermically prior to the gassing which was carried out at a concentration of 50 parts in 10,000 of air.

The heart maintained a rapid rate until the time of respiratory failure. Following this, the rate slowed, the P-R time increased and a-v block developed, but without the stage of auricular cessation noted in the unatropinized animals. The dying heart ended in ventricular fibrillation.

In one of the animals, a short period of alternation involving both the R and T waves developed at the time of greatest respiratory augmentation. This is illustrated in figure 15. Figures 14 and 16 precede and follow this respectively by periods of 5 minutes. Neither gives indications of alternation.

The development of the deviation in the direction of the T wave, as noted previously, was unaffected by atropine.

Protocol 3 summarizes the findings from an experimental asphyxia preceded by atropine injection.

The heart after long exposure to carbon monoxide. The length of time during which the animal is subjected to deprivation of oxygen is a factor in bringing about pathological changes quite as important as the degree of anoxemia. For the investigation of this point, three

Protocol 3. Experiment 9. Carbon monoxide asphyxia after administration of atropine ($\frac{1}{100}$ gr.). Dog 9, female, 14 kilos. Gassed in 50 parts of carbon monoxide in 10,000 of air

TIME	REMARKS	ELECTROCARDIOGRAPHIC RECORD
min.		
0	Atropine given	
5	Record taken and gassing started	Rate 143 per minute. P–R interval 0.14 second.
10	Respiration rapid and somewhat jerky	Rate 193 per minute. P–R interval 0.14 second. T wave increased in height
15	Respiration greatly augmented	Rate 272 per minute. (Fig. 14.)
20	Respiration gasping	Rate 214 per minute. Alternation in height of T and R waves. P–R interval 0.14 second. (Fig. 15.)
25	Occasional gasping	Rate 167 per minute. P–R interval 0.14 second. No evidence of alternation. (Fig. 16.)
26	Respiration stopped	Rate 152 per minute. P–R interval 0.14 second.
27		Rate 152 per minute. P–R interval 0.16 second.
28		Rate 96 per minute. P–R interval 0.16 second.
29		Rate 76 per minute. P–R interval 0.20 second.
30		3:1 block auricular rate 62 per minute. P–R interval 0.30 second.
31		4:1 heart block auricular rate 48 per minute. P–R interval 0.28 second.
33		Auricle stopped. Ventricular complex bizarre; 42 per minute.
35		Ventricular complex very bizarre; 31 per minute.
36		Ventricular fibrillation.

dogs were gassed with 25 parts of carbon monoxide in 10,000 of air until death occurred. This covered periods of 5, $6\frac{1}{2}$ and 7 hours. Electrocardiographic tracings were taken at intervals. No evidence of impairment of cardiac conduction developed until respiration had failed. The picture presented was essentially similar to that of animals under rapid asphyxia. The development of heart block, however, occurred and passed off somewhat more quickly. Ventricular fibrillation developed more quickly after respiratory failure than it did in those animals with shorter asphyxia. Protocol 4 is a typical experiment of this type.

In the gassings covering periods of several hours compensatory changes develop that tend somewhat to neutralize the time element.

Protocol 4. Experiment 14. Dog 14. Male, 6 kilos. Gassed in 25 parts of carbon monoxide in 10,000 of air

TIME	REMARKS	ELECTROCARDIOGRAPHIC RECORD
min.		
0	Normal record. Gas started	Rate 109. P–R interval 0.14 second.
60	Noticeable augmentation of respiration	Rate 164. P–R interval 0.14 second.
120	Extremely labored respiration	Rate 208. P–R interval 0.14 second. T wave increased in height.
180		Rate 220. P–R interval 0.12 second.
240	Cheyne-Stokes respiration	Rate 216. P–R interval 0.12 second. Slight respiratory arrhythmia. T wave diphasic.
300		Rate 196. P–R interval 0.14 second. Numerous ventricular extra-systoles. T wave inverted.
360		Rate 186. P–R interval 0.14 second. Ventricular extra-systoles.
390	Respiration gasping and irregular	Rate broken by ventricular extra-systoles.
410	Great respiratory embarrassment	Respiratory arrhythmia. Auricular inhibition during respiratory pause. P–R interval 0.16 second.
415	Respiration stopped	Rate 52 per minute. P–R interval 0.21 second.
416		Rate 52 per minute. P–R interval 0.31 second.
417		3:1 block. Bizarre ventricular waves. Auricle 46 per minute.
418		Complete block. Auricular rate 26 per minute. Ventricular rate 42 per minute.
419		Auricle stopped. Bizarre ventricular waves 28 per minute.
420		Ventricular waves 16 per minute.
421		Bizarre ventricular waves 46 per minute.
422		Bizarre ventricular waves. Irregular 74 per minute.
423		Ventricular fibrillation.
425		Ventricular fibrillation.

The heart during recovery. Three dogs were gassed rapidly as in the first series, electrocardiographic tracings being taken at intervals. When a condition of advanced respiratory embarrassment was reached the animals were removed from the gassing chamber. A short period of manual artificial respiration was necessary to prevent death and the animals were then allowed to recover. At intervals electrocardiographic tracings were taken.

The cardiac rate remained rapid during recovery and even after the animal was apparently completely normal again, 3 or 4 hours later. The T wave continued in the direction opposite to the normal after a lapse of 4 hours following gassing. In all three of the animals the T wave had assumed its normal position at the end of 24 hours and all evidence of tachycardia had passed.

Three other animals were permitted to pass into the stage of complete respiratory failure and remain so until partial heart block had developed. The attempt was then made to resuscitate the animals by means of a treatment recently devised in this laboratory (7). It consists in inhalation of 8 or 10 per cent CO_2 in oxygen, initially under artificial respiration, and then under spontaneous respiration stimulated by the CO_2. Recovery was successful with two of the animals. Electrocardiograms could not be taken during the vigorous breathing induced by the CO_2 in the inhalation. But after termination of the inhalation when the animal had quieted sufficiently, after 10 to 18 minutes, to allow an electrocardiographic record to be taken, all evidence of impairment of conduction had passed off. One animal exhibited a normal curve with only a moderate tachycardia and rather pronounced respiratory arrhythmia. The second animal which had passed to a somewhat greater stage of heart block (1–4) showed numerous ventricular extrasystoles still persisting 2 hours after the recovery. The animal's later condition was not followed, but there was no reason to doubt complete recovery.

Asphyxia when respiration is sustained by CO_2. The extent to which the impairment of conduction was due to cessation of respiration was investigated by preventing respiratory failure by means of inhalation of 7 per cent carbon dioxide. At the moment of removal from the gassing chamber the two animals used had respectively 82 and 84 per cent of the hemoglobin in combination with carbon monoxide. They were replaced in the chamber in an atmosphere containing carbon monoxide plus carbon dioxide. Respiration continued actively, and the absorption of carbon monoxide progressed to a higher degree than has ever otherwise been observed in this laboratory. One animal died after 14 minutes due to cardiac inhibition. Administration of atropine would undoubtedly have prevented this. The heart's blood showed 91 per cent of the hemoglobin combined with carbon monoxide. The second dog was removed after an exposure of 12 minutes with 93 per cent of its hemoglobin combined with carbon monoxide. This animal died 6 minutes after removal from the chamber apparently from respi-

ratory failure, presumably from inadequate CO_2. Unfortunately no tracing was obtained at this time. While in the CO_2 atmosphere neither animal showed any evidence of impairment of a-v conduction; for respiration was maintained under this stimulus.

The evidence from these experiments indicates clearly that the impairment of cardiac conduction under carbon monoxide is purely due to anoxemia. Carbon monoxide exerts no direct toxic action on the heart.

The experiments show another point of great interest, in that these two animals reached a percentage saturation with carbon monoxide much higher (6 to 15 per cent) than has ever occurred in this laboratory when carbon monoxide in air was used. Evidently the presence of carbon dioxide maintains respiration, prolongs life, and thus under the conditions above described increases the degree of carbon monoxide saturation.

Asphyxia wtih illuminating gas. It has been noted by Henderson and Haggard (7) that animals asphyxiated with illuminating gas (coal gas plus fattened water gas) diluted in air, die at a lower percentage saturation of hemoglobin with carbon monoxide than do animals exposed to a comparable concentration of pure carbon monoxide.

Electrocardiographic and pneumographic tracings were taken from animals gassed in illuminating gas diluted with air to a concentration of 50 parts of carbon monoxide in 10,000.

The course of events was essentially the same as that found under rapid asphyxiation with pure carbon monoxide of like concentration. There was, however, a much greater augmentation of respiration, and respiratory failure occurred at an earlier stage. The period of auricular cessation was slightly longer than in the animals exposed to pure carbon monoxide; the development of delayed conduction and a-v block was slower; and a greater time elapsed between the failure or respiration and the final cessation of cardiac activity or development of ventricular fibrillation.

Evidently the accessory toxic substances in illuminating gas exert a stimulating action upon respiration and thus hasten the development of acapnia, and respiratory fatigue and failure. But during short exposures there is no indication that these substances are directly and rapidly toxic. The delay in development of heart block may be accounted for by the greater oxygen supply (less carbon monoxide saturation) at the time of respiratory failure.

CONCLUSIONS

Death under carbon monoxide asphyxia is due to failure of respiration. This is in the nature of a fatal apnoeavera. The anoxemia resulting from the formation of carboxyhemoglobin induces excessive breathing; and respiratory failure follows the excessive loss of CO_2.

Oxygen deficiency caused by carbon monoxide, even in advanced asphyxia is not in itself sufficient to cause impairment of auriculo-ventricular conduction. Following respiratory failure, however, the increased anoxemia from this cause speedily results in the development of heart block through its various stages.

By restoring respiration and rapidly eliminating the carbon monoxide by means of inhalations of carbon dioxide and oxygen, cardiac conduction is restored to normal following the development of block.

The cardio-inhibitory center maintains its activity longer than does the respiratory center. This center is stimulated by the increased C_H which occurs during respiratory failure. From this there results a temporary cessation of auricular activity. This period of inhibition is prevented by the administration of atropine.

When respiratory failure is prevented by means of inhalations of 8 or 10 per cent carbon dioxide, the carbon monoxide combination with hemoglobin rises to an unusually high percentage without any evidence of impairment in a-v conduction. This indicates that there is no direct toxic action of carbon monoxide upon the cardiac conducting system.

Illuminating gas results in an earlier development of respiratory failure than does pure carbon monoxide in corresponding concentration.

Electrocardiographic records are given from two animals which differed from the rest in that one developed a transient period of alternation involving the R and T waves and the other presented, during the time of complete a-v block, a condition resembling auricular fibrillation or flutter.

In conclusion I wish to express my sincere thanks to Prof. Yandell Henderson for his advice and suggestions made during the course of this work.

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STUDIES IN NUTRITION

VIII. The Nutritive Value of the Proteins of Tomato Seed Press Cake

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Received for publication March 22, 1921

Large quantities of tomato[1] seeds and skins are at the present time discarded. These are by-products occurring in the manufacture of tomato catsup, soups and pastes. Rabak (1) has estimated that 1500 tons of dried tomato seeds could be obtained annually in this country. Tomato seeds contain approximately 22 per cent of a valuable oil suitable for table use (2), (3), (4). The press cake which remains after expelling the oil contains about 37 per cent of protein (N × 6.25). This has been used to some extent as a fertilizer and also as a cattle feed.

The isolation and chemical analysis of the proteins from the tomato seed are in progress in this laboratory. Preliminary experiments indicate the presence of two globulins differing chiefly in their nitrogen and sulphur content (5).

The nutrition experiments described in this paper with tomato seed press cake[2] show that its proteins are efficient for the normal growth of albino rats, and that the press cake is as valuable a protein concentrate as peanut, soy bean or cocoanut press cake.

Tomato seed press cake may also contain some fat-soluble vitamine, since normal growth was obtained on a diet in which the butter fat, which is usually incorporated to provide this vitamine, was replaced by an equivalent amount of lard. There is still some question, how-

[1] *Solanum esculentum.*

[2] The tomato seed press cake was furnished by J. H. Shrader, formerly with the Bureau of Plant Industry, U. S. Department of Agriculture. The seeds, previous to expelling the oil, were dried in direct heat rotary dryer at a temperature which did not exceed 60°C. The resulting press cake was ground to a meal which was incorporated in the rations used in our experiments.

ever, whether or not lard may be a source of fat-soluble vitamine. Daniels and Loughlin (6) have reported successful experiments in which lard was the only apparent source of fat-soluble vitamine in an otherwise complete diet. In a recent publication by Osborne and Mendel (7) it was shown that 28 per cent of lard, carefully rendered at a low temperature did not contain enough fat-soluble vitamine for normal growth. To explain these divergent results, Osborne and Mendel (7) offered the following tentative hypothesis:

"Must we assume for the present either that the lard from different sources varies in its content of fat-soluble vitamine, or that ether alone fails to extract it from the non-fat foodstuffs?"

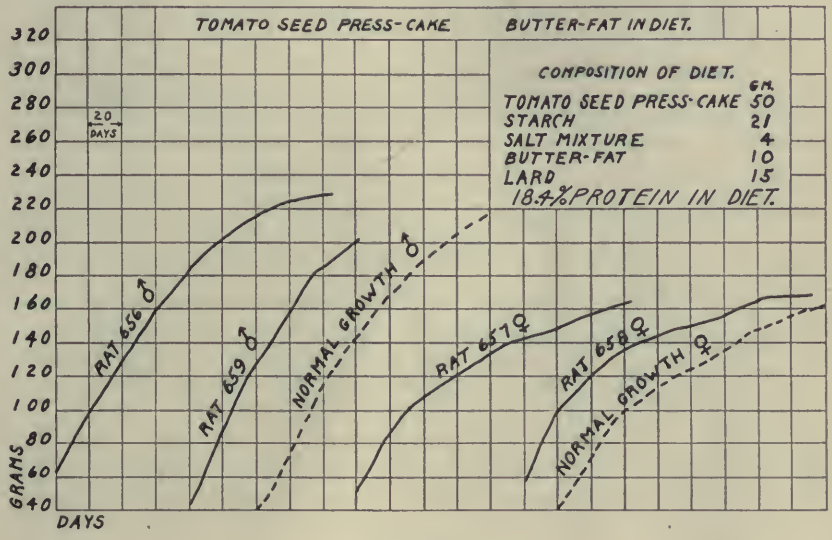

Chart 1

Drummond and his co-workers (8) have shown that the diet of the pig, part of the body from which the pig fat is taken, as well as the process of lard manufacture, all influence the fat-soluble vitamine content of the finished product.

It is also interesting to note that many of the nutrition experiments in which the proteins were biologically adequate, viz., soy bean (9), (10), peanut (11), (12), cotton seed (13), (14), (15), (16), and cocoanut (17), as in the case with the tomato seed, were also oil bearing seeds.

Experimental. A diet consisting of 50 parts of tomato seed press cake, 21 parts of commercial cornstarch, 15 parts of lard, 10 parts of

butter fat and 4 parts of the Osborne and Mendel salt mixture, when fed to albino rats, resulted in the normal rate of growth. These experiments indicate that tomato seed press cake contains a sufficient quantity of water-soluble vitamine for normal growth. Such a diet contained 18.4 per cent of protein. The results of these experiments are recorded on chart 1.

A similar diet, with the exception that the 10 parts of butter fat were replaced by an equivalent amount of lard, was also adequate for nor-

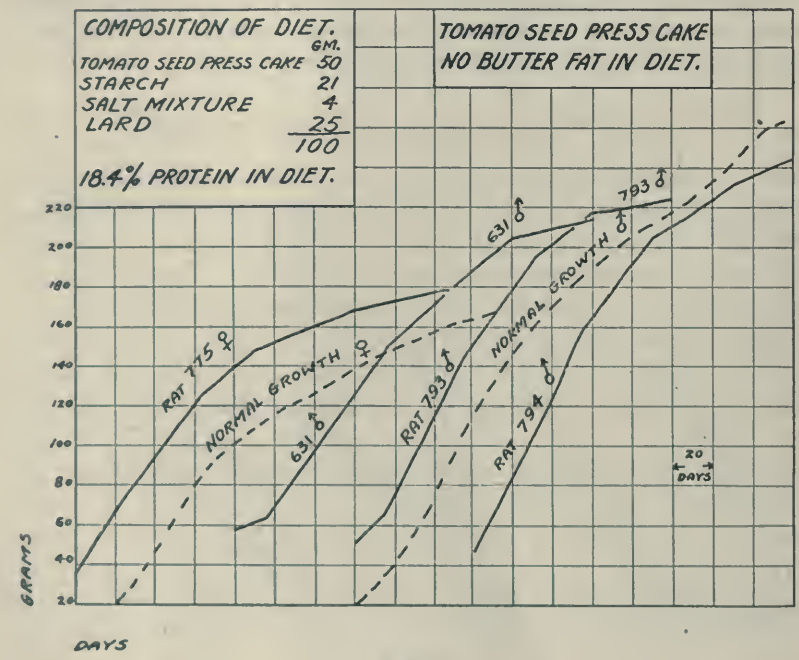

Chart 2

mal growth. The question of whether or not the residual oil in the tomato seed press cake or the press cake itself contained enough fat-soluble vitamine must be left in abeyance until it is definitely shown that the other dietary constituents are not sources of fat-soluble vitamine. In this connection it is interesting to note that Osborne and Mendel (18) found that 0.1 gram of dried tomato, furnished daily, contained enough fat-soluble vitamine for normal growth. The ether extract of dried tomato, however, was not potent and failed to promote renewal of growth in rats that had declined on a diet which was deficient in fat-soluble vitamine.

The results of our experiments are shown on chart 2.

SUMMARY

A diet in which tomato seed press cake furnished the sole source of protein and water-soluble vitamine enabled albino rats to grow at the normal rate. Such a diet was made adequate by the addition of starch, a suitable inorganic salt mixture, butter-fat and lard. Normal growth was also obtained when the butter fat of the above diet was replaced by lard.

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THE MODE OF ACTION OF LOW TEMPERATURES AND OF COLD BATHS IN INCREASING THE OXIDATIVE PROCESSES

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Received for publication March 24, 1921

As a result of the work of Lavoisier (1) and of a great number of investigators (2) since his time, it is now known that a fall in the external temperature as well as cold baths increase oxidation in warm-blooded animals and decrease it in cold-blooded animals. The action of cold on the skin of warm-blooded animals produces reflexly an increased innervation of the muscles resulting in movements (shivering) or in the increase in tone. Several observers (3) have shown that unless the lowering of the temperature or the coldness of the bath is sufficient to cause shivering or muscular tension, no increase in oxidation results. Cold-blooded animals, on the other hand, have no heat regulating mechanism such as is found in warm-blooded animals and hence their heat production rises and falls with a rise and fall in external temperature.

The increase in heat production brought about by cold in warm-blooded animals is usually attributed to the additional heat produced by the action of the muscles. Voit believed that the increase in metabolism brought about by cold was due to a reflex stimulation of the muscles resulting in an increase in the inherent power of the muscle cells to metabolize.

We (4) had already found that whatever increased oxidation in the body, the ingestion of food, for example, brought about an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide, by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation, narcotics, for example, produced a decrease in catalase by decreasing its output from the liver and by direct destruction. Hence we naturally turned to catalase for an explanation of the increase in oxidation brought about by exposure of warm-blooded

animals to cold and for the decrease in oxidation on exposure of cold-blooded animals to low temperatures.

The warm-blooded animals used were dogs and the cold-blooded, turtles. The effect on the blood catalase of keeping these animals in a cold chamber as well as in hot and cold baths, and in baths at different temperatures was tried out.

The cold chamber was constructed large enough to accommodate a dog comfortably. A stream of fresh air was forced through the chamber continuously. Further provision was made for the animal to get fresh air by cutting a small window in the wall of the cold chamber through which the animal could poke its snout. The temperature of this chamber could be kept fairly constant over a period of several hours. Catalase determinations were made of the blood of the jugular before as well as at intervals after the dogs were placed in the chamber. The determinations were made by adding 1 cc. of the jugular blood to 50 cc. of neutral hydrogen peroxide in a bottle and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the blood. The normal catalase content of the blood was established by making determinations on two or three successive days previous to performing the experiment.

The following experiment will illustrate the method as well as show the effect of cold on the blood catalase in vivo. A short-haired, adult dog weighing about 3 kilos was placed in the chamber at 2°C. and permitted to remain there for 3 hours. One cubic centimeter of the jugular blood of this dog prior to being placed in the chamber liberated 74 cc. of oxygen from 50 cc. of neutral hydrogen peroxide in 10 minutes. After he had been in the cold chamber for 3 hours, a similar amount of jugular blood liberated 100 cc. of oxygen from hydrogen peroxide in 10 minutes. The dog was then kept in a room at 22°C. for 17 hours and at the end of this time the catalase of the blood had returned to normal, that is, 1 cc. of the blood liberated 70 cc. of oxygen from hydrogen peroxide. This experiment was repeated using the same dog with comparable results.

The catalase content of the jugular blood of another short-haired dog, older and much fleshier than the preceding, was determined and 1 cc. of his blood was found to liberate 250 cc. of oxygen. After being in the chamber for $1\frac{1}{2}$ hours, his blood liberated 278 cc. of oxygen and after 3 hours 293 cc. On keeping the dog for about 17 hours in a room at a temperature of 22°C. the blood catalase returned to normal.

E. Voit (5) showed that the metabolism of the pigeon may be doubled by removing its feathers. Rubner (6) found that the metabolism of a dog was greatly increased by the clipping of his hair. The following experiment was carried out using a very long-haired poodle dog to determine if cold would produce a greater increase in the blood catalase after his hair was clipped than it did before. One cubic centimeter of the jugular blood of the long-haired poodle dog was found to liberate 226 cc. of oxygen from neutral hydrogen peroxide in 10 minutes. The dog was kept in the cold chamber for 3 hours at 2°C. and at the end of this time it was found that the blood catalase had been increased by about 7 per cent. After the blood catalase of the dog had returned to normal, his hair was clipped and he was again placed in the chamber at 2°C. for 3 hours. It was then found that the cold increased the catalase of the jugular blood by about 16 per cent. By comparing these results, it may be seen that the percentage increase produced after the clipping was more than twice as great as it was before the clipping.

The following experiment was carried out to determine the effect on the blood catalase of exposing the same dog at different temperatures for the same length of time. Three catalase determinations were made using the jugular blood, one before the dog was placed in the cold chamber, another after he had been in the chamber for 3 hours at 2°C. and a third, 17 hours later during which time the dog was kept in a room at 22°C. Similarly catalase determinations were made after the dog had been kept in the cold chamber at 7°C., 15°C. and 22°C. respectively. The results of the determinations are shown in figure 1. The figures along the ordinate indicate percentage increase in catalase and those along the abscissa, time in hours.

It may be seen that the exposure of the dog for 3 hours in the cold chamber at 2°C. increased the catalase of the blood 35 and 36 per cent respectively in two experiments; that exposure at 7°C. increased it 18 per cent; exposure at 15°C. increased it 15 per cent and at room temperature (22°C.) practically no change in the catalase content of the blood was produced. It may be seen further that 17 hours after each experiment during which time the animal was kept in a room at 22°C., the blood catalase had returned to practically normal in all cases.

Rubner (7) and others have shown that cold baths greatly increase the oxidative processes; that this effect decreases with a rise in the temperature of the bath, disappearing entirely at 35°C. and again is increased at 44°C. The increase in heat production brought about

by cold baths is usually attributed to the additional heat produced in shivering, and that by the very hot baths to the warming of the cells.

The following experiments were carried out to determine the effect of very cold baths (3°C.) and baths at different temperatures on the blood catalase. The animals used were dogs. Approximately 200 liters of water were used for the bath. The dog, with the exception of his head and neck, was immersed in the water. There was very little struggling on the part of the animal. Upon removing from the bath, the animals were rubbed dry with a cloth and placed in a warm chamber at 30°C. In a short time the dog was warm and comfortable.

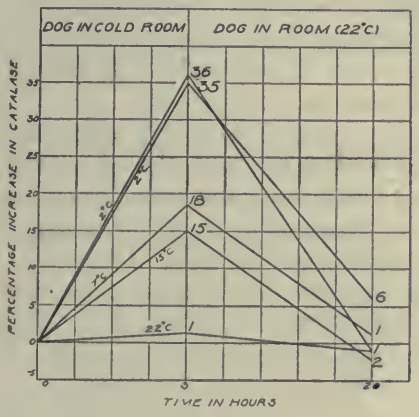

Fig. 1

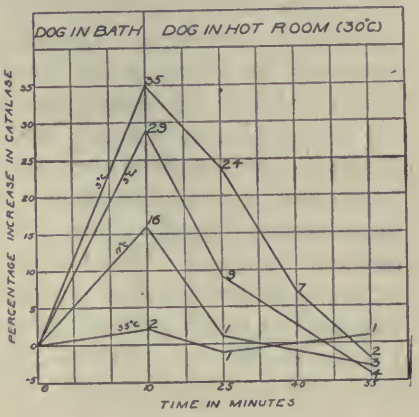

Fig. 2

Fig. 1. Curves showing the effect on the blood catalase of exposing the same dog to different degrees of cold.

Fig. 2. Curves showing the effect of baths at different temperatures on the blood catalase of the same dog.

Catalase determinations were made using the jugular blood of a medium-sized, short-haired dog. It was found that 1 cc. of the blood liberated 198 cc. of oxygen in 10 minutes from 50 cc. of neutral hydrogen peroxide. The dog was placed in the cold bath (3°C.) for 10 minutes. At the end of this time 1 cc. of the blood liberated 268 cc. of oxygen from hydrogen peroxide. The dog was rubbed dry and placed in the warm chamber. Fifteen minutes later 1 cc. of the blood liberated 246 cc. of oxygen; 30 minutes later, 212 cc. and 45 minutes later, 1 cc. of the blood liberated practically the same amount of oxygen as it did before the dog was placed in the cold bath, that is, the blood catalase had returned to its normal amount. Similar experiments were carried out using several different dogs with similar results.

The following experiment was carried out to determine the effect of baths at different temperatures on the blood catalase, using the same dog. The results are shown in figure 2. It may be seen that a bath at 3°C. for 10 minutes increased the blood catalase 35 and 29 per cent respectively in two experiments, the same dog being used; that a bath at 17°C. for 10 minutes increased the blood catalase 16 per cent; and a bath at 35°C. produced practically no change in the catalase content of the blood. It may be seen further that the blood catalase had returned practically to normal 45 minutes after the baths.

It is known that oxidation is increased in cold-blooded animals when the external temperature is raised and is decreased when the external temperature is lowered. The following experiments were carried out to determine the effect on the blood catalase of cold-blooded animals of raising and lowering the external temperature.

The cold-blooded animals used were turtles (Pseudemys concinna). Catalase determinations were made according to the method described in the first part of this paper. The blood used was taken from the aortic arches through a hole 2 cm. in diameter made in the plastron just over the heart. This hole was kept closed by means of a cork.

Several determinations were made using the same turtle which had been kept at ordinary room temperature (22°C.) and it was found that 1 cc. of blood liberated 790 cc. of oxygen in 10 minutes from 200 cc. of neutral hydrogen peroxide. This turtle was packed in crushed ice for 15 hours and at the end of this time 1 cc. of the blood liberated 270 cc. of oxygen from neutral hydrogen peroxide. One cubic centimeter of blood from another turtle was found to liberate 760 cc. of oxygen from neutral hydrogen peroxide in 10 minutes. This turtle was placed in warm water at 40°C. for 30 minutes. Upon removal from the warm water 1 cc. of the blood liberated 850 cc. of oxygen. Experiments similar to the preceding were carried out with comparable results.

The following experiment was carried out using the same turtle to determine the effect on the blood catalase of exposing the animal to cold as well as to heat. The animal was kept in a warm bath at 40°C. and later packed in crushed ice for 15 hours.

It may be seen in figure 3 that 1 cc. of the blood of the turtle while being kept at ordinary room temperature (22°C.) liberated 760 and 765 cc. of oxygen respectively from 200 cc. of neutral hydrogen peroxide in 10 minutes and that after keeping this turtle in a hot bath (40°C.) for 30 minutes the amount of oxygen liberated by the blood increased to 850 cc. It may be seen further that on keeping the turtle for the

following 6 hours at room temperature, the blood catalase returned to normal, that is, 1 cc. of blood liberated 765 cc. of oxygen from hydrogen peroxide in 10 minutes. On keeping the turtle packed in crushed ice for 15 hours, the blood catalase was greatly decreased, 1 cc. of blood

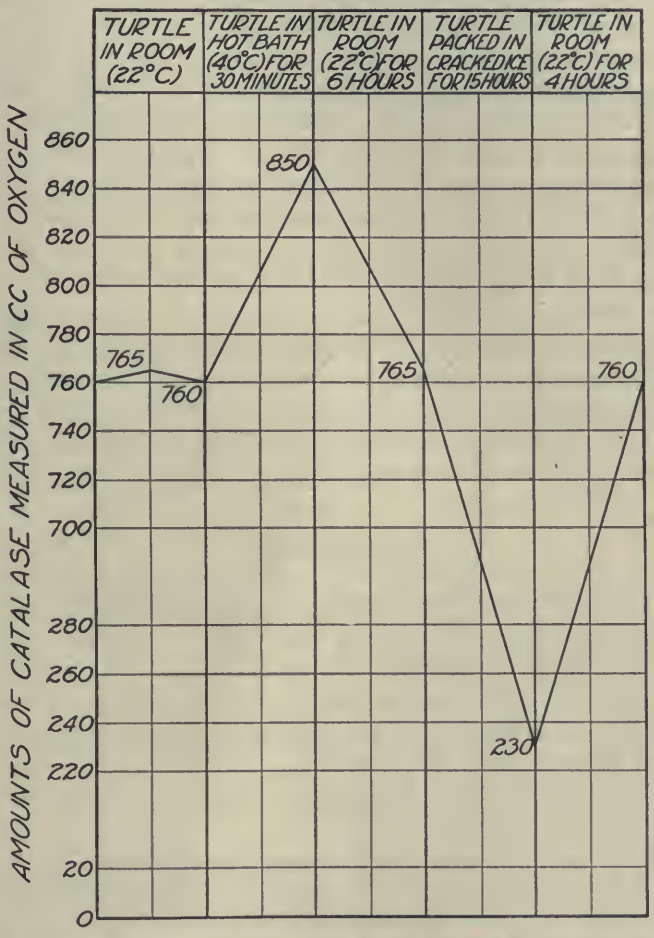

Fig. 3. Curves showing the effect of high and of low temperatures on the blood catalase of the same turtle.

liberating only 230 cc. of oxygen, and that 4 hours later, during which time the animal was kept at room temperature, the blood catalase had returned to normal. It should be said in this connection that the turtle had also become normal so far as its activity was concerned.

SUMMARY

Low temperatures and cold baths produce an increase in the blood catalase of warm-blooded animals and a decrease in cold-blooded animals, in keeping with the fact that cold increases oxidation in warm-blooded animals and decreases it in cold-blooded animals. An increase in the external temperature increases the blood catalase in cold-blooded animals in keeping with the fact that it increases the oxidative processes.

The stimulating effect of low temperatures on catalase production in warm-blooded animals decreases with a rise in temperature, disappearing at room temperature (22°C.) in keeping with the fact that the stimulating effect of cold on metabolism decreases with a rise in temperature. Baths at 35°C. produce no increase in oxidation in warm-blooded animals and it is shown in this paper that such baths do not increase catalase.

The increase in oxidation in warm-blooded animals on exposure to cold is attributed to an increase in catalase, and the decrease in oxidation in cold-blooded animals on exposure to cold, to a decrease in catalase. The increase in oxidation in cold-blooded animals occurring with a rise in external temperature is attributed to an increase in catalase.

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STUDIES ON THE CONSECUTIVE PHASES OF THE CARDIAC CYCLE

I. The Duration of the Consecutive Phases of the Cardiac Cycle and the Criteria for their Precise Determination

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Received for publication March 26, 1921

During the course of investigations pertaining to the behavior of the heart under certain experimental pathological conditions, it became evident that the compensatory phenomena of the ventricles involve not only changes in the *vigor* of the ventricular contractions but alterations also in the *duration* of certain phases of its contraction and relaxation processes. In order to determine how far these mechanisms are normally operative in adapting the work of the heart to differing normal conditions and how far they are held in reserve until abnormal conditions arise, it became necessary to establish how the duration of each consecutive phase of the cardiac cycle can be altered in length under normal conditions. This not only involves a subdivision of the periods of systole and diastole into still shorter phases but also necessitates the establishment of precise criteria for the accurate calculation of the length of these consecutive phases.

METHODS

An analysis of the consecutive phases of the cardiac cycle can be most successfully accomplished by a study of pressure curves optically recorded from the cardiac chambers and vascular system. During the course of my work, extending over a period covering the last five years, a total of about 6 kilometers of tracings showing simultaneous pressure curves from two portions of the vascular system have accumulated. These records include curves obtained under normal as well as various experimental conditions produced in 192 different dogs. In addition to this unusually large amount of material, a further series

of 18 experiments was recently added in which three simultaneous pressure curves from the auricle, ventricle and aorta were simultaneously recorded. In these experiments, in which the heart within the pericardium was exposed, three optical manometers of essentially the same type as described by the writer (1) in 1914, were inserted as follows: One into the left ventricular cavity through the muscular wall, a second into the left auricle through the tip of the auricular appendage and a third, via the innominate artery, into the aorta so that its tip lay near the aortic valve. Each manometer was rigidly fixed by clamps so that no movements of the light beams occurred when the cannulae stopcocks were closed.

In endeavoring to establish precise time relations by the use of several curves simultaneously recorded by such optical projections, certain difficulties arise. In the first place, attention must be paid to the shifting of recorded points due to parallax. Methods of obviating such errors have been discussed by C. Tigerstedt (2), Garten (3), and lately again by Straub (4). For various reasons, the methods suggested for obviating such displacement or for correcting it could not be employed in this work. The following arrangement was used: Each manometer was illuminated by a separate arc light, the only source of illumination still available which is capable of producing satisfactory curves on bromide paper moving more than 100 mm. per second. For anatomical reasons only one manometer (usually the ventricular) could be so arranged that the reflection center of the mirror was directly in line with the camera lens. The other manometers, i.e., the auricular and aortic, were faced away from the photokymograph and the incident beams projected on their mirrors were reflected to two other "first surface" mirrors which were vertically superimposed and so arranged that the light centers were in direct line with the vertical camera slot. When care was taken in the alignment, subsequent photographic checks on stationery paper showed no displacement of synchronous points due to parallax. Needless to say, such checks were always made and appropriate corrections carried out when needed.

A second difficulty arises in the evaluation of the time differences due to a delay of pressure transmission within the vascular system. In the case of comparisons between arterial and ventricular curves, for example, such correction has usually been made by measuring the distance of the arterial cannula from the aortic orifice and calculating the delay on the basis that the pressure wave travels with a velocity of

approximately 7 meters per second. Such corrections can not be considered very exact. In the first place, the velocity of the pressure transmission changes during the cardiac cycle, being modified, at least, by the momentary changes in the volume elasticity coefficient. This is illustrated in figure 3, in which the delay in transmission of several points of the pressure curves is shown. The upper curve was taken from the innominate artery, 6 cm. from the semilunar valves; the lower curves represent pressure changes quite close to the valves. If we estimate the delay at obviously similar points such as *D* and *H*, it will be found that the delay at point *D* equals 0.0187 second, whereas at point *H* it is equivalent to 0.0034 second. Estimating the velocity on the basis of post-mortem measurements above noted, it is found that the velocity of pressure propagation is 3.2 meters per second at the point *D*, and 17.6 meters per second at point *H*.

Furthermore, there is great difficulty in measuring with exactness the distance between the aortic orifice and the arterial cannula, partly because it is difficult to be quite certain as to the precise plane that functionally separates aorta and ventricle; partly also because the length of the aorta apparently changes with the fullness of the vessel and the changing position of the heart during experimental work. It is my impression that post-mortem measurements can not be made with sufficient exactness for use in time corrections involving a few σ.

It is therefore not surprising that various careful investigators, such as Piper (5), C. Tigerstedt (2) and Garten (6), each believing his technic unimpeachable, should have arrived at somewhat different interpretations as to synchronous events in the several pressure curves. Since it is probably as yet not possible always to rely implicitly on time corrections made in this way, all interpretations must be tempered by a consideration as to whether the dynamic events thus indicated are possible. While synchronous events on different curves can be established without difficulty in such records as are used to illustrate this paper, it must be frankly acknowledged that I have many tracings in my possession in which adherence to strict corrective processes, such as outlined above, would force one to admit that a condition of dynamic chaos must exist in the cardiovascular mechanisms, and one could point out many dynamic absurdities in published records of other investigators were one inclined to be critical.

SUGGESTED SUBDIVISIONS OF THE CARDIAC CYCLE

The term *systole* is generally used to designate the period of muscular contraction and the term *diastole*, the combined period of physiological relaxation and any possible additional phase of quiescence. This division of the cardiac cycle has proven so useful in studying the cardiac cycle that it seems desirable to adhere to it. A further subdivision of these two *periods* has, however, been suggested, on separate occasions, by different investigators. To promote clarity these will be referred to as *phases* of systole and diastole in this article.

Subdivision of systole. The earlier work of Chauveau and Marey, Hürthle and V. Frey suggested the subdivision of ventricular systole into two phases: an Anspannungszeit and Austreibungszeit. The latter phase has been usually referred to as the ejection phase by English writers but it has been more difficult to find a concise and accurate term by which to designate the Anspannungszeit. The phrase "period of rising tension" is too long and the term "presphygmic period" is unfortunately inaccurate since, as Frank (7) first showed, there is a well-marked sphygmic oscillation during this phase. Frank (8) has also pointed out that the dynamic reactions of the ventricle closely correspond to those of an after-loaded skeletal muscle which contracts first isometrically and later isotonically. On this basis I suggested the term isometric phase in place of the period of rising tension. Frank has also definitely pointed out, however, that during the ejection phase, the ventricle, unlike an after-loaded skeletal muscle, is not contracting under an absolutely constant tension and that it therefore contracts only in an approximately isotonic fashion. Starling (9) proposed the term auxo-tonic (that is, acting under an increasing tension) as expressing the nature of ventricular contraction during the ejection phase. This phrase, however, is also not quite descriptive of the dynamic conditions of the normal heart which contracts first against an increasing but later against a decreasing load. If a general term is desirable, the phrase "allasotonic" (i.e., acting under a variable tension) would seem more appropriate. On the whole, however, it will be just as well perhaps to refer to the two phases of ventricular systole as the isometric and ejection phases. In the light of other investigations, it is necessary to consider the advisability of further subdivision. Thus, Piper's interpretation of the optical ventricular pressure curve (5), supported also by the work of Dean (10) and my own analysis of the pressure curves, indicates that the a-v valves are

not closed effectively until a slight elevation of intraventricular pressure has taken place. If this is the case, then the period rising tension may include a pre-isometric as well as an isometric phase. So also it is possibly necessary to divide the ejection phase into an early phase of maximum ejection and a later phase of reduced or absent ejection. Frank (8) has directed attention to the fact that the aortic pressure rises during the early portion of ejection and falls during the latter portion of this phase because the peripheral outflow from the arterial system exceeds the ventricular volume ejected into the aorta during this latter phase. This can only mean that the ventricle must eject a smaller quantity of blood toward the end of systole. Such a reduced ejection is manifest also in normal volume curves reported by Henderson (11), de Heer (12) and Straub (13), and according to Patterson, Piper and Starling (9) such curves may show "some justification for the old idea that the latter portion of ventricular systole represents a 'Verharrungszeit' or 'Rückständage Contraction,' " i.e., a condition in which the ventricles remain contracted without expelling any blood.

Phases of diastole. On the basis of pressure curves Hürthle (14) subdivided diastole into a phase of rapidly declining tension and a diastolic inflow phase. de Heer (12) points out that the former represents a phase in which the ventricle relaxes with all valves closed, i.e., isometrically. Accordingly, the interval between the closure of the semilunar valves and the opening of the a-v valves might be designated as the isometric-relaxation phase. According to the volumetric studies of Henderson and his co-workers (11), the inflow, starting at the opening of the a-v valves, does not continue with equal rapidity until the end of diastole, provided the period of diastole is sufficiently long; on the contrary, there appears a phase of relative stasis or diminution in the rate of blood flow from auricle to ventricle. This interval they have designated as diastasis and originally added as a third major period of the cardiac cycle. For reasons already indicated, it seems preferable to consider this as a third phase of diastole.

More recent analyses of the pressure and volume curves of the ventricles indicate that the phase of declining tension mentioned by Hürthle must be further subdivided. Finally, the experiments of Straub (13), Patterson, Piper and Starling (9), and also those of Gesell (15) indicate that auricular contraction affects the dynamics of ventricular efficiency sufficiently to warrant its inclusion as a last phase of ventricular diastole. As these suggested divisions of the cardiac cycle are not as yet universally accepted, we are not only confronted with the task of

determining the demarcation of the several consecutive phases of the ventricular cycle but are compelled also to inquire into the necessity, as well as the desirability, of these suggested subdivisions.

THE CONSECUTIVE PHASES OF THE VENTRICULAR CYCLE, AS INDICATED BY THE PRESSURE CURVES

The consecutive changes in pressure occurring within the left auricle, left ventricle and root of aorta of a vigorously beating heart are shown in figures 1 and 2. The technical data are given in the legends. As a basis for initiating analysis, five lines are drawn to indicate the customary subdivisions that have been used by others and myself in analyzing the cardiac cycle. Lines *1–2* demarcate the interval usually designated as the isometric contraction phase; *2–3* limit the ejection phase; 3–4 comprise the early diastolic relaxation phase; *4–5* include the rapid inflow phase and the balance of the cycle comprises the phase of diastasis terminating in auricular systole. In considering the time limits of these phases, the desirability of further subdivision will be discussed.

The demarcation of the isometric phase. If, as in figures 1 and 2, we consider the isometric phase of contraction as beginning with the onset of the pressure rise, we are at once confronted with the questions as to whether the a-v valves are closed prior to ventricular systole and, if not, whether the isometric contraction may be regarded as beginning at this point.

The idea that the a-v valves are closed, not by the elevation of intraventricular pressure following ventricular systole but by the cessation of a jet of blood forced into the ventricle during auricular systole, appears to have been first suggested by the observations of Baumgarten (16) in 1843. This investigator showed that when a stream of water directed against the valves is suddenly stopped, they immediately float into a position of closure so that the heart may be inverted without leakage of fluid through them. These observations have been confirmed by Henderson and Johnson (17) in the case of valves mounted as naturally as possible in a glass beaker. While these experiments leave no doubt that the auricular and ventricular valves *can* float into apposition by the sudden interruption of a vigorous stream, it does not necessarily follow that a similar mode of closure occurs in the beating heart in which the force of auricular systole must be very much less and the dynamic conditions are somewhat different. Direct records of the movements of the valvular cusps in a beating heart recorded by Dean

(10) indicated that while the force of auricular systole is indeed capable of floating the valves toward a position of closure, a complete approximation accompanied by sound vibrations apparently does not take place unless a subsequent ventricular systole follows.

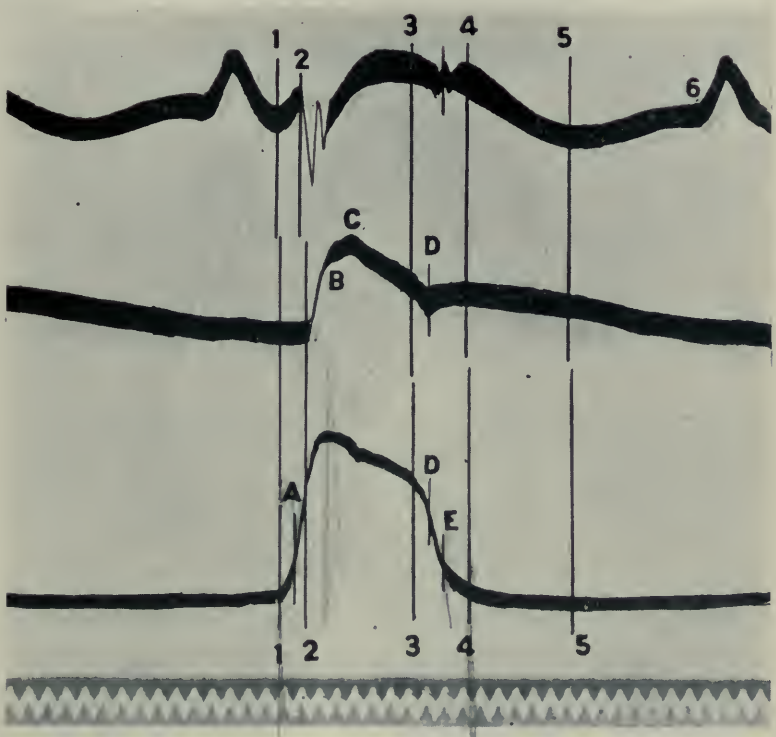

Fig. 1. Synchronous records of intra-auricular pressure (upper); pulmonary arterial (middle); right ventricular pressure (lower). Tuning fork 0.02 second; *1–2*, isometric contraction phase; *2–3*, phases of maximum and reduced ejection; *3–D*, proto-diastolic phase; *D–4*, isometric relaxation phase; *4–5*, rapid inflow phase; *5–6*, diastasis. Further description in text.

The chief argument advanced by those who incline to the theory of pre-systolic closure seems to be that, in such cases, a slight regurgitation of blood into the auricle must inevitably result unless the valves are closed before ventricular systole begins. The writer has not been able to follow clearly the logic involved. Henderson and Johnson, for example, liken the regurgitation to the volume of air displaced from a room into a corridor when a door closes by a hinge movement. Whether

such a displacement should be considered as a regurgitation in the sense that the term has come to assume, depends, of course, on whether the door frame or the door itself is considered as the line of division between the room and the corridor. In any case, no considerable volume could be displaced auricle-ward, but such a displacement as occurs must cause some elevation of auricular pressure. Are there any indications on the pressure curve that this takes place? Even a casual inspection

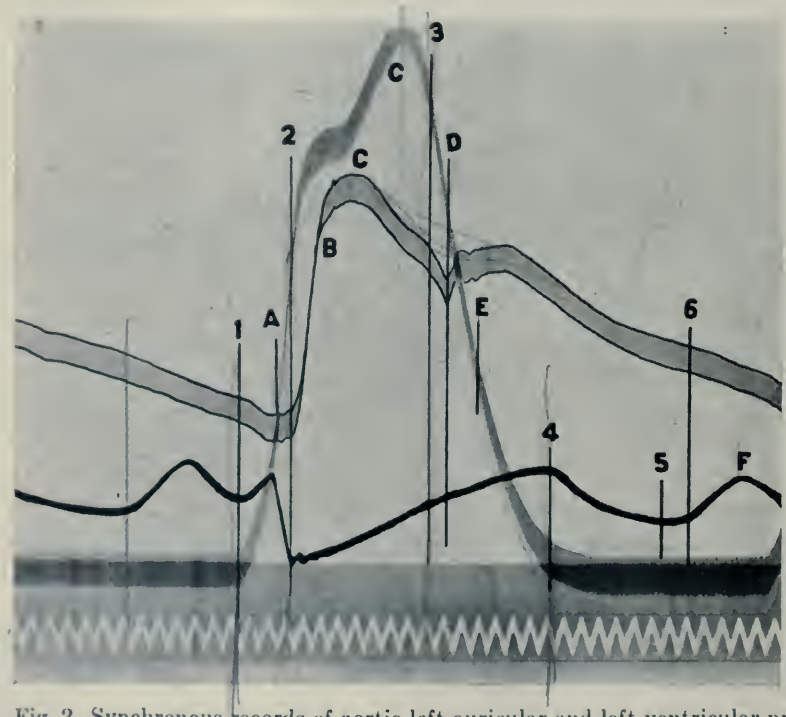

Fig. 2. Synchronous records of aortic left auricular and left ventricular pressures. Letters same as in figure 1.

of the auricular pressure curves such as are shown in figures 1 and 2, indicates that the intra-auricular pressure rises synchronously with or very slightly after the first elevation of intraventricular pressure. These and similar elevations shown also in the published curves of Piper (5), as well as in those of Garten and Weber (18), might readily be accounted for by such a regurgitation. Close inspection of the intraventricular pressure curves also indicates that the pressure increases in two stages, first slowly, *1-a*, then suddenly changing to a steeper

incline, *a-2*. This change in the rate of tension development may, of course, be attributed to various causes. Thus, de Heer (12) points out that in such a gradual beginning, it resembles the isometric tension curve of a skeletal muscle, and believes that it represents the time required for the development of a concerted muscular contraction. Fahr (19), without much warrant, interprets a similar rise in Garten's curves as due to the earlier contraction of the papillary muscles. Piper (5) explains this rise as due to a loss of energy during the closure of the a-v valves, and is inclined to believe that a point somewhere near *1-a* represents the moment of complete closure of the a-v valves. The further observation reported by Dean and myself (20) that the amplitude of the first heart sound increases at this point would favor such an interpretation.

The bulk of recent work tends to favor the idea that final and complete closure of the a-v valves is not produced until some elevation of intraventricular pressure has taken place. It may at once be pointed out, however, that this does not preclude the incorporation of this short interval in the isometric contraction phase for, during this interval of time, the valves are undoubtedly in the process of closure and the orifice must be exceedingly small. Consequently, during this brief interval, the ventricular contraction must be so nearly isometric in character as to make it undesirable to further subdivide this phase of contraction.

Having settled upon the initial elevation of intraventricular pressure as the beginning of the isometric phase, we may consider the other criteria that correspond to this point. Wiggers and Dean (20) have shown that the *first* maximal vibration of the first heart sound begins synchronously with the first elevation of the pressure rise. Consequently, this point may be taken as coincident with the elevation of intraventricular pressure and the beginning of the isometric phase. Is the beginning of the isometric phase also indicated on the arterial pressure records from the aorta and central arteries? In a detailed analysis of the central arterial pulse recorded from the subclavian or innominate artery, Frank (7) observed that a small preliminary vibration (2te Vorschwingung), such as is marked *C-D* in figure 3, usually precedes the primary rise in pressure. This wave he interpreted as occurring during the isometric phase. Subsequent work, however, indicates that the beginning of this wave does not definitely mark the very onset of systole. In the pulmonary arterial pressure curves the writer (2) usually found two such vibrations and similar double vibrations are shown in the arterial pressure curves published by Piper (5)

and Garten (6). These investigators, moreover, showed that such double vibrations begin a few σ after the beginning of ventricular contraction, relations that are confirmed in my tracings (cf. fig. 2). Frank further observed that when arterial pressure is low, this preliminary vibration is preceded by a negative dip similar to the depression *B-C* shown in figure 3. Such a depression is quite constantly present in pulmonary arterial pressure curves and not infrequently in optical arterial pulse tracings taken from man. These experiments indicate that this negative depression, rather than the positive wave following, coincides with the beginning of the intraventricular pressure rise. When present, this depression may therefore be taken as the beginning of systole. Unfortunately, however, it is so inconstant and its beginning so difficult to determine, that such tracings can not be relied upon in calculating the beginning of the isometric contraction phase.

The phase of isometric contraction ends with the beginning of the ejection phases. This point is indicated definitely by the sharp rise of aortic pressure (figs. 1 and 2) but its location on the ventricular pressure curves has been much discussed. According to Piper's interpretation (5), the intraventricular pressure curve shows, at the moment of ejection, a small notch topping the sharp rise of intraventricular pressure; while C. Tigerstedt (2) and Garten (6) both believe that this notch, when it occurs, is synchronous with the primary wave of the aortic pulse. My own results are in agreement with those of the latter investigators and indicate that the transition from the isometric to the ejection phases occurs without interruption of the intraventricular pressure rise or is indicated at most by a very slight change in the gradient (e.g., fig. 2). It follows that the intraventricular pressure curves, on the whole, do not show a sufficiently clear criterion for determining the end of the isometric contraction phase. Nor do minor variations in intra-auricular pressure bear a constant relation to the end of this phase. Thus, in figure 2, the sudden drop of intra-auricular pressure occurs precisely with the rise of aortic pressure, but in figure 3 it appears to occur somewhat earlier.

Conclusion: The onset of the isometric contraction phase may be accurately obtained from the initial rise of intraventricular pressure, from the onset of the first sound vibration, and sometimes though less surely, from a depression preceding the initial vibration in the arterial pressure curves; the end of the isometric contraction phase can be determined only by the rise of aortic pressure.

The ejection phase and its subdivision. The ejection phase obviously begins where the isometric contraction phase ends. As a matter of convenience, Hürthle placed its end at the point where the semilunar valves have closed, which would be at the bottom of the incisura of the intra-arterial pressure curve (figs. 1 and 2, *D*). Inasmuch as the beginning of a muscular relaxation is definitely indicated by the beginning of the incisura, it seems preferable to consider this the end of the ejection phase. Accordingly it is possible, as in figures 1 and 2, to

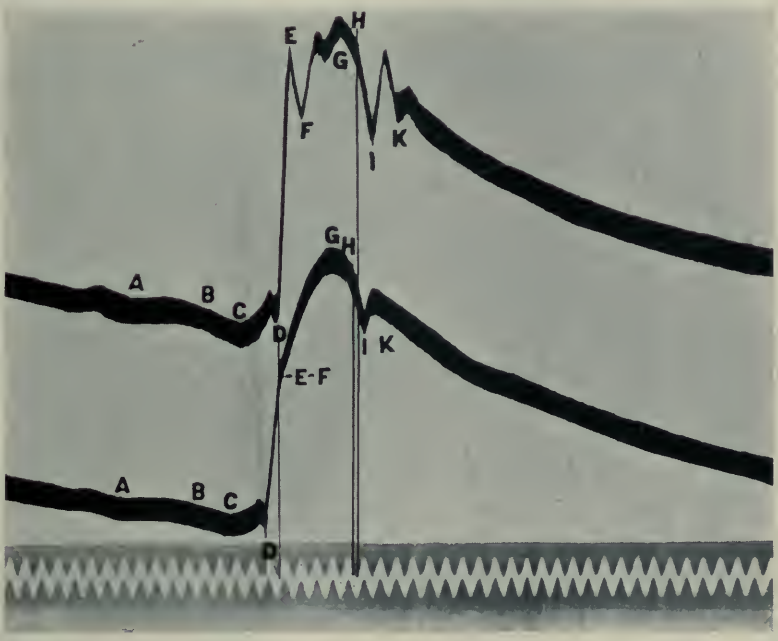

Fig. 3. Synchronous records of pressures in aorta near semilunar valves (lower) and in innominate artery (upper). Showing the addition of systolic vibrations in central arteries. Letters referred to in text.

include the ejection phase by the lines *2* and *3* and consider the pressure variations in the cardiac chambers and large arteries.

The optical tracings recorded by O. Frank (7) from the subclavian and innominate arteries show several variations during the ejection phase (cf. also fig. 3). The pressure change is initiated by a large primary vibration, *D E F*, reaching a prominent peak. This is followed by a more gradual rise to a summit, *G*, and lastly by a gradual decline to *H*. In the root of the aorta, near the semilunar valves,

these variations are all present except the pronounced primary vibration, *D E F*, which is much reduced, according to the records published by Piper (5), C. Tigerstedt (2) and Garten (6). That this is not determined by experimental conditions is clearly proven by synchronous records of the aortic and subclavian pulse, a specimen of which is shown in figure 3. It is quite apparent that this vibration is intensified as the rapid pressure change is transmitted to the aortic branches. The summit of the aortic pressure curve (figs. 1, 2 and 3, *F*) may come early or late in the ejection phase, the time depending, according to O. Frank, on how rapidly a balance is struck between systolic ejection and the peripheral flow from the arterial system. Whenever, therefore, the arterial pressure does not continue to increase until the end of systole, the assumption may safely be made that the rate of ventricular ejection has decreased and the summit may be used as the point separating the early phase of maximal ejection from the later phase of reduced ejection.

Before discussing the relation this bears to the mode of ventricular contraction, it is necessary to briefly consider the changes of intraventricular tension during these phases. Inasmuch as aorta and ventricle are a common cavity during the entire phase of ejection, it may be anticipated, on *a priori* considerations, that the contours of the ventricular and aortic pressure curves are similar and that the times of their summits coincide. This is frequently the case, as is shown in figure 1, and also in many published tracings of C. Tigerstedt (2) and Garten (6). It was therefore quite startling to find that in fully 50 per cent of my tracings such correspondence does not obtain. Figure 2 is really used as an extreme illustration of such discrepancy in the two curves. Obviously, the pressure summit, *C*, is reached quite early in the aortic curve, while it is greatly delayed in the intraventricular curve. In fact, while the aortic pressure is declining, the intraventricular pressure is rising. The suggestion has therefore occurred to me that, under certain experimental conditions of the circulation, a functional stenosis, due to the systolic approximation of the semilunar valves, may supervene to produce such effects. Less blood entering the aorta would account for the aortic pressure decline; an increased retention would, likewise, explain the simultaneous elevation of intraventricular tension. Attention is directed to these instances in order to impress the fact that a reduced discharge of the ventricle toward the end of systole is not necessarily due to a reduction in contractile energy or to an inadequate volume of blood for expulsion but may possibly also arise from an accidental functional valvular phenomenon.

Conclusion: The ejection of blood may be considered as occurring in two phases, the maximum ejection phase and the reduced ejection phase. The time occupied by these phases may be computed most accurately from the aortic pressure curves, the maximum ejection phase extending from the beginning of the steep pressure increase in the aorta to the summit of the curve; the reduced ejection phase extending from the latter point to the beginning of the incisura.

The early diastolic fall in pressure and its subdivision. With the beginning of ventricular relaxation, several consecutive events follow in rapid sequence: the semilunar valves close, the a-v valves open and then blood rushes into the ventricle. When the latter event begins, the early diastolic fall in pressure is nearly completed. As this inflow of blood is definitely accompanied by a fall of intra-auricular pressure, we may consider the lines *3–4* (figs. 1 and 2) as limiting this phase.

In marking the beginning of this phase as taking place at the moment when systole ceases (e.g., *3*, figs. 1 and 2), we include the time occupied by the closure of the semilunar valves, *3-d*. As already pointed out, Hürthle and many who have followed him include this interval in the period of systole. There can be no doubt, however, that ventricular contraction ceases at *3* and that strictly this is not a post-systolic phase. Is it, however, a proto-diastolic event? Has the left ventricle actually begun to relax? What actually happens is this: with the cessation of ventricular contraction at *3*, the pressure at once falls in the ventricle and communicating aorta. The backward movement of blood, indicated in the aortic incisura, probably brings into apposition the semilunar valves which have already floated toward a position of closure. Not until such closure has been effected, however, does a steep decline of intraventricular pressure, such as one might logically associate with a relaxation process in muscle, take place. It is quite probable therefore that the passing of the ventricle from a state of systole to that of diastole is not a momentary event but is represented rather by this time interval required for the closure of the semilunar valves. In this sense the interval belongs, strictly speaking, neither to the period of systole nor to that of diastole. Since we would be unwarranted on the basis of such a hypothetical analysis and its extremely short duration in considering this as an intermediary period of the ventricular cycle, we are forced to add it either to the period of systole or to that of diastole. The former has usually been done. Since all evidence points to the end of muscular contraction previous to this point, however, and since the phase of diastole already includes

other events than those associated with actual relaxation processes in the muscle, I believe it most logical to consider this as a proto-diastolic rather than a post-systolic event.

As already indicated, the duration of the proto-diastolic phase is measured by the time required for the arterial fall in pressure designated as the incisura *3-d*. A similar drop of pressure may be present in the intraventricular pressure curve. Here, however, the end alone is distinct, the beginning being, as a rule, difficult to place. The intra-auricular pressure curve shows no landmarks by which the duration of this phase can be directly determined.

The time interval existing between the complete closure of the semilunar valves and the opening of the a-v valves, *D-4*, represents the isometric relaxation phase. It is determined most accurately from the combined aortic and auricular pressure curves. During this phase of isometric relaxation, the pressure at first falls abruptly (fig. 1, *D-E*) and then more gradually (fig. 1, *E-4*). Attention has been directed to this change in gradient by Garten (6), who offers no explanation as to its cause. In previous publications I have interpreted the change in gradient as coincident with the inflow of blood from the auricle, i.e., have interpreted this to represent the end of the isometric relaxation phase and the beginning of the rapid diastolic inflow phase. On the basis of Piper's curves (5) and also my own, this interpretation appears incorrect. Obviously, a great transfer of blood from the ventricle to the auricle does not occur until the auricular pressure drops, a short time after (4). Tachograms of the ventricle recently reported by Straub (13), (21) led him to believe that about this time the a-v valves bulge more rapidly toward the ventricle previous to opening. This may be the explanation of the change in pressure gradient. The end of the isometric relaxation phase is therefore not clearly indicated on the intraventricular pressure curve itself.

Conclusion: The early fall of ventricular pressure constitutes two diastolic phases: a proto-diastolic phase, the length of which is indicated by the duration of the incisura of the arterial pressure curve, and an isometric relaxation phase extending from the bottom of the incisura to the fall of auricular pressure coincident with the onset of ventricular filling.

The phases of rapid diastolic inflow and diastasis. According to the ventricular volume curves of Henderson (11), the ventricles, under normal conditions of venous pressure, fill within a short time interval after opening of the a-v valves. If the cycle is long, this filling comes

to an abrupt end due to an equalization of auricular and ventricular pressures. The first stage may well be designated as the phase of rapid inflow; the second, during which a gradual filling occurs, as *diastasis*. Naturally, as Henderson and his collaborators point out, the stage of diastasis is non-existent whenever the total period of diastole does not exceed the time taken for the rapid inflow. Is this subdivision of diastolic inflow justifiable? Not all physiologists hold that the ventricle fills in this manner but, on the contrary, believe that approximately the same rate of ventricular inflow persists throughout diastole. This idea is based on the interpretation of ventricular volume curves recorded by Straub (21), Patterson, Piper and Starling (9), Socin (22) and others. It is not proper at this time to enter into a discussion as to the cause of these discrepancies. We may, however, at present approach the subject from another angle, viz., the evidence supplied by synchronous pressure curves. If a period of stasis normally supervenes in long cycles, we may anticipate that the intra-auricular pressure recorded by sensitive manometers will rise subsequent to its diastolic decline, whereas if a rapid inflow of blood into the ventricles continues throughout diastole, no such increase should be observed. That the latter condition is frequently found is apparent from tracings published by Piper and also by Garten. In my own tracings about 28 per cent of the left auricular pressure curves showed a definite increase such as is evident in figure 1. In these cases, at least, we must infer a distinct phase of diastasis. It is possible to interpret the failure of the auricular pressure rise in the other 72 per cent of cases as showing that ventricular inflow into the ventricles continues with sufficient rapidity so that no rise of auricular pressure takes place. Even if such a phase is present only in a minority of experiments, it seems desirable to retain this subdivision of diastole into phases of *rapid inflow* and *diastasis*.

Conclusion: Since the ventricular inflow in a certain proportion of cases is sufficiently interfered with to cause an elevation of auricular pressure, it seems preferable to subdivide the filling periods of the ventricle into a phase of rapid inflow extending from the fall of auricular to the subsequent rise of auricular pressure (i.e., *4–5*) and to consider the remainder of diastole up to the beginning of auricular systole, *5–6*, as a phase of diastasis.

The "auricular-systole" phase of diastole. Considerable difference of opinion has also developed as to the efficacy of auricular systole in filling the ventricles. Henderson (11) believes that the auricular contraction contributes only an insignificant increment, while Straub (13),

(21), Patterson, Piper and Starling (9), as well as Gesell (15) hold that ventricular filling is helped in this way. As a more comprehensive analysis of this question is anticipated, we shall restrict our present evidence to the pressure curves. If auricular systole injects a considerable quantity of blood into the ventricle, we may anticipate an elevation of intraventricular pressure. As Frank (7) pointed out, such a wave is frequently found in the aortic pressure curve which he interprets as a transmission of a similar intraventricular variation. Previously published optical curves of different investigators give instances in which such an auricular wave is present in the ventricle. In my large collection of normal records it is present in only 13 per cent. In many cases, as shown in figure 1, the wave may be entirely absent from the intraventricular curve yet distinctly indicated by the intra-aortic curve, which makes it probable that Frank's explanation of the latter is incorrect and that it owes its origin to a direct communication of pressure from the auricle. Again, it is desirable to take cognizance of such a phasic event in the cardiac cycle since it is present in some cases. Such recognition requires that we go further, however. Auricular systole is capable of exerting a dynamic effect on the ventricle—either increasing its volume or elevating the initial tension to which fibers are submitted—only so long as the intra-auricular pressure continues to rise. In a previous communication (23) I have pointed out that auricular pressure rises only during the early half of its systole and that, before systole is over, it may have declined to the pressure level existing before its onset. This primary elevation of auricular pressure alone causes a flow of blood ventricleward, while during the decline of auricular pressure due to the passing off of certain fractionate contractions blood actually enters the auricle from the veins. We may therefore divide auricular systole into an early Dynamic Interval and an Inflow Phase.

THE DURATION OF THE CONSECUTIVE PHASES OF THE CARDIAC CYCLE IN THE DOG

The duration of each successive phase of the cardiac cycle of normal dogs was established in 38 carefully controlled experiments. Two classes of these experiments were carried out, viz., *a*, those in which the animal was placed under light morphine and chloretone anesthesia and the thoracic cavity left intact; and *b*, those in which the heart was exposed but the pericardium and its attachments left intact. In the former experiments, right auricular and subclavian pressures were

optically recorded together with the heart sounds; in the latter, curves of left auricular, left ventricular and aortic pressures were taken. From these records the successive phases summarized in the diagram of figure 4, were calculated according to the criteria above analyzed.

The margin of variation in consecutive heart beats. When the duration of the separate phases of systole and diastole are estimated by accurate methods, small variations in the duration of many phases occur from beat to beat. These are attributable partly to normal modifications of the heart beat, partly perhaps to unavoidable errors or personal factors introduced in measuring curves. It is therefore necessary for any experimenter to evaluate the combined factors before lending significance to variations which may occur under modified conditions of the circulation.

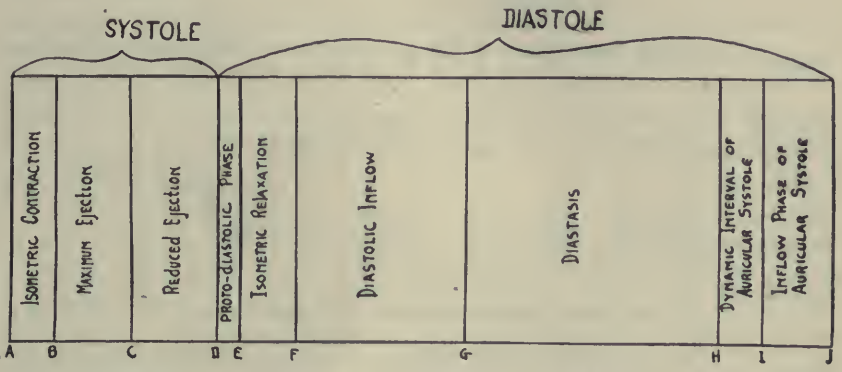

Fig. 4. Scheme showing consecutive phases of cardiac cycle

For this reason the duration of each successive phase in each of 15 consecutive cardiac cycles was first personally calculated and tabulated. From 38 of these tabulations a second table was made which showed the margin of variation that existed in each phase (table 1). A study of such tables containing values for about 1000 cardiac cycles showed the following *maximal variations* in 15 consecutive cycles of normal anesthetized animals; the actual variations in most series of heart beats being of course very much smaller: Isometric contraction phase, 0.008 second; maximum ejection phase, 0.01 second; reduced ejection phase, 0.022 second; proto-diastolic phase, 0.01 second; isometric relaxation phase, 0.01 second; early diastolic inflow phase, 0.045 second; diastasis phase, variable; auricular systole, 0.027 second (table 1). Changes in the duration of any phase during experimental procedures

in excess of these variations may therefore be safely considered as *prima facie* evidence of an alteration arising in consequence of the altered circulatory conditions.

The influence of opening the thorax and of cardiac exposure. In view of the fact that the cardio-dynamics are certainly altered in many

TABLE 1

The duration of consecutive phases of the cardiac cycle in dogs

	ISOMETRIC CONTRACTION	MAXIMUM EJECTION	REDUCED EJECTION	TOTAL SYSTOLE	PROTO-DIASTOLE	ISOMETRIC RELAXATION	RAPID INFLOW	DIASTASIS	AURICULAR SYSTOLE	TOTAL DIASTOLE
A. In exposed hearts (22 experiments)										
Range	0.042–0.078	0.050–0.130	0.031–0.142	0.149–0.305	0.012–0.038	0.050–0.112	0.022–0.104	0.000–0.246	0.044–0.078	0.184–0.494
Average when possible	0.045–0.050				0.025	0.060–0.070	0.080		0.070	
Variation*	0.000–0.006	0.003–0.010	0.003–0.022		0.001–0.010	0.000–0.010	0.001–0.009			
B. In closed chest experiments (16 experiments)										
Range	0.038–0.054	0.050–0.110	0.063–0.144	0.148–0.260	0.015–0.035	0.04 –0.090	0.040–0.130	0.000–0.540	0.062–0.120	0.184–0.496
Average when possible	0.048–0.050				0.022	0.048–0.050	0.045–0.060		0.085	
Variation*	0.000–0.008	0.000–0.010	0.000–0.012		0.000–0.005	0.001–0.010	0.000–0.045		0.027	

*The two figures refer to the least and greatest variations found in 15 consecutive cycles taken in the same animal.

respects when the chest is opened, it becomes necessary to analyze what effect such experimental procedures exert on the lengths of the successive phases of the cardiac cycle. The results alluded to above and summarized in table 1 were obtained from 22 animals in which the thorax was left intact and from 16 animals in which the heart was exposed. The two series of results show a fortunate correspondence

as to ranges both of heart rate and lengths of systole and diastole, making it possible to compare data as to variations of the smaller phases.

Considering the results, phase by phase, it is evident that the isometric contraction phase in the case of exposed hearts shows practically the same variations as that of the enclosed heart. The durations of the maximum ejection phase and reduced ejection phase, both absolute and relative, show variations, however, that are distinctly greater in the exposed heart. This indicates that the adaptation of the ejection rate to the peripheral resistance is altered to varying degrees in different operated animals. This is precisely what might be expected for the amount of blood lost in opening the thorax, and possibly also the early vascular reactions of shock incident to the operative procedures is naturally variable in the case of different animals. Furthermore, the value of the respiratory pressor factor (i.e., the assistance offered by respiratory movements to the filling and emptying of the heart) is not constant in different animals, consequently its elimination may operate to affect the duration of the phases of maximum ejection more in some animals than in others.

Passing to the diastolic phases, we note a remarkable correspondence in the duration of the proto-diastolic phases, which it should be recalled represent the times required for the semilunar valves to float into apposition. If anything, this phase is slightly greater in the case of the exposed heart. The predominant duration of the isometric relaxation phase is longer in the exposed heart by fully 0.02 second. The phase of early diastolic inflow varies greatly both in closed and open chest experiments, but the predominant length is again longer in the case of the exposed heart. Whether this indicates that the negative intrathoracic pressure assists the isometric relaxation and rate of rapid inflow when the chest is intact can not be definitely stated. The fact must be borne in mind that the data from open chest experiments were derived by the aid of left auricular tracings, those from closed chest experiments from right auricular tracings. We are not yet in a position to state conclusively that the two ventricles always and necessarily begin to fill at the same time.

In considering the great variation in the duration of the early diastolic inflow phase, we must, of course, consider the possibility that this phase might have been prematurely terminated in some cases by the onset of the next systole. In other words, variations in this phase are only of vital significance when the heart is slow and a phase of diastasis can

exist. It was found in the 38 experiment studies that there were 14 cases in which a possible diastasis phase could exist. In 12, or 85 per cent, a distinct indication of a diastasis phase, such as is shown in figure 1, was shown. In only two cases the fall of auricular and presumably a rapid rate of ventricular inflow continued until the onset of the next auricular systole. In these 12 experiments in which the evidence of a diastasis phase is present, the early diastolic inflow phase varies from 0.044 to 0.13 second, the prevailing period in closed chest experiments being 0.045 to 0.06 second. In the open chest experiments the prevailing duration is somewhat longer, i.e., approximately 0.088 second. We may therefore infer that 0.045 second is the very minimum time interval during which the heart can fill rapidly after opening of the a-v valves and that in many cases this interval is much longer.

TABLE 2

Comparative results as to duration of diastolic phases

PHASES	HENDERSON (11)	DE HEER (12)	AUTHOR'S RESULTS	
			Open chest	Closed chest
Proto-diastolic plus				
A. Isometric relaxation	0.077	0.025	0.085	0.072
B. Rapid inflow	0.052	0.135	0.08	0.06
Total ($A + B$)	0.129	0.15	0.165	0.132
Auricular systole	0.110	0.085	0.07	0.08

Physiological significance of results. One of the mooted questions of cardiodynamics is that of the manner in which diastolic filling of the ventricles takes place. The ventricular volume curves of Henderson (11) and de Heer (12) indicate that, after short proto-diastolic and isometric relaxation phases, ventricular filling follows rapidly. After this the inflow rate is greatly reduced, i.e., a period of diastasis supervenes which continues until the next auricular systole follows. In the following table is given a comparison of the data calculated from the typical reconstructed volume curves of Henderson (11) and de Heer (12) together with some actual figures obtained from these experiments as to the relative lengths of the diastolic phases exclusive of diastasis (table 2).

These comparisons render it obvious that (with the exception of a wrong calculation by de Heer for the proto-diastolic and isometric relaxation periods, which correspondingly lengthens the early diastolic inflow phase) the results are in as close agreement as could logically be

expected. This is of significance for it indicates that, as far as temporal relations are concerned, the heart relieved of its pericardial support and enclosed within a plethysmograph relaxes and fills like the heart in the intact chest.

As previously stated, the view that the heart fills as indicated by Henderson and his collaborators has not been supported by other investigators who believe that filling continues rapidly throughout diastole, and that no phase of diminished inflow or diastasis exists. Henderson has repeatedly pointed out, by clear and emphatic logic, that the failure of such records to evidence a diastasis phase is due to a rapid heart rate and the consequent shortening of the total diastolic length. On the basis of my results we may calculate how short total diastole must become in order to have the phase of diastasis disappear entirely. Let us add together what, on the basis of my results, must admittedly be conservative intervals for the following diastolic events:

	Second
(a) Proto-diastolic phase	0.022
(b) Isometric relaxation phase	0.05
(c) Early diastolic inflow phase	0.06
(d) Auricular systole	0.085
Total	0.217

It is obvious that unless diastole exceeds 0.217 second, no phase of diastasis is possible; if it is shorter, the early diastolic inflow phase must also be encroached upon by auricular systole.

Let us, however, make another calculation of the following events:

	Second
Proto-diastolic phase	0.022
Isometric relaxation phase	0.05
Auricular systole	0.085
Total	0.157

It is obvious that the phase of early diastolic filling must be entirely absent when total diastole is reduced to about 0.16 second, consequently ventricular filling then occurs only during auricular systole. Owing to the fact that systole and diastole vary independently in length, it is not possible, I believe, to accurately translate these figures into terms of heart rate. It is probable, however, that whenever the heart rate increases to 180, auricular contractions come during the early diastolic inflow phase, while at the rate of 200, ventricular filling must come

entirely during auricular contraction. I take this occasion to reëmphasize by definite figures what Henderson and his collaborators have so clearly demonstrated by the use of volume curves, because it seems difficult to drive home the idea that such records as are obtained from rapid hearts can supply no critical proof of what obtains in the filling of hearts at rates that may be regarded as quite normal for man.

SUMMARY

An analysis of optical pressure curves from a total of over 200 dogs, in 38 of which special pains were taken to maintain normal conditions, leads to the following interpretation of the consecutive phases of the ventricular cycle:

1. At the beginning of ventricular systole, the a-v valves have been partly floated into apposition, probably in consequence of the sudden cessation of a jet when the peak of the intra-auricular pressure curve is reached about the middle of auricular systole. The first elevation of intraventricular pressure firmly closes these valves and the ventricle then contracts absolutely isometrically. *This first phase of ventricular systole extending from the beginning of the pressure rise until the opening of the semilunar valves is preferably considered as the isometric contraction phase.* The predominant durations of this phase range from 0.048 to 0.05 second when the chest is intact, and from 0.045 to 0.05 second when the heart is exposed.

2. As soon as intraventricular exceeds intra-aortic pressure, the semilunar valves open and a comparatively large volume of blood is ejected. As long as the volume ejected remains greater than the outflow from the peripheral arterioles, the aortic pressure continues to rise. This marks a *second phase of maximum ejection.* As soon, however, as the systolic ejection decreases enough so that it no longer equals the peripheral outflow, then the aortic pressure begins to decline. This *third phase* when present may be designated as the phase of *reduced ejection.* This terminates the period of systole. The absolute and relative durations of these phases vary considerably, the greatest variations in closed chest experiments being 0.05 to 0.11 second for the maximum ejection phase and 0.063 to 0.144 second for the reduced ejection phases.

3. When ventricular relaxation begins, aorta and ventricles are still in communication. The first event, viz., the closing of the semilunar valves, is signalled by a sharp drop in aortic and intraventricular pressure, designated as the incisura. This marks the *fourth* or *proto-diastolic*

phase. This phase is relatively constant averaging 0.022 second in closed chest experiments and 0.025 second when the heart is exposed.

4. Following closure of the semilunar valves and until the a-v valves have opened, the ventricle relaxes without any flow of blood either from or into its cavity. This marks the *fifth phase* or that of *isometric relaxation.* The predominant duration of this phase in exposed hearts is 0.06 to 0.07 second but is predominantly shorter in animals with thorax intact, ranging from 0.048 to 0.05 second.

5. With the opening of the a-v valves and until either an equalization of pressure between auricle and ventricle has taken place or a subsequent systole interrupts the filling, a rapid filling of the ventricles takes place. This is the *sixth phase,* conveniently designated as the *rapid inflow phase.* This phase averages from 0.048 to 0.05 second in closed chest experiments and from 0.06 to 0.07 second in exposed hearts.

6. In long cycles and when the auricular pressure is normal, a period of reduced filling obtains which may be designated, after Henderson's suggestion, as the *phase of diastasis.* Its duration is obviously dependent on the length of total diastole.

7. Finally there may be added an *eighth phase* during which the dynamic interval of auricular systole affects the filling or pressure of the ventricles. This does not continue until the end of auricular systole, however, but a terminal phase of auricular systole in which no dynamic effect can be exerted exists before the entire cycle is completed. The predominant durations of the total phase of auricular systole average 0.07 second in exposed hearts and 0.085 second in hearts enclosed within the thorax.

A pictorial summary of these dynamic events occurring during ventricular systole and diastole is schematically indicated in figure 4.

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STUDIES ON THE CONSECUTIVE PHASES OF THE CARDIAC CYCLE

II. The Laws Governing the Relative Durations of Ventricular Systole and Diastole

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Received for publication March 26, 1921

While the relation existing between the lengths of systole and diastole have been quite extensively studied under a variety of conditions, both in animals and man, the fundamental laws which determine the extent and direction of these variations under different circulatory conditions have not been clearly formulated.

Perhaps the most definite existing basis upon which to attempt such a formulation of governing laws rests upon the interpretation given the ventricular volume curves by Henderson and his collaborators (1). These investigators, it will be recalled, pointed out that under normal conditions of venous pressure both ventricular filling and discharge occur after a constant pattern, exemplified by a record of the ventricular volume changes. According to their interpretation, the extent of ventricular filling and discharge under normal conditions can be determined solely by changes in the length of preceding diastoles. It was recently pointed out by Katz and myself (2) that it becomes a necessary corollary of such a conception that the durations of systole and diastole at any heart rate must bear a fixed relation to each other, which relation we believe it is possible to derive from an animal and express in a curve form. Actual experimental observations showed, however, that these relations are not maintained when the heart is accelerated either by stimulation of the accelerator nerves or after the administration of adrenalin. Subsequently Katz (3) also directed attention to the fact that during the course of vagal slowing or during subsequent recovery from such slowing, these relations do not hold good. Our former observations may be interpreted in part at least as indicating a specific effect of the accelerator nerves on the ventricles,

but the latter effect certainly is more probably interpreted as, in some way, associated with alterations either in venous pressure or arterial resistance (3). That changes in venous return or arterial resistance may have a direct determining effect on the length of systole is also indicated in several reports from other laboratories. Thus Lombard and Cope (4), finding that the systoles of the left ventricle are longer in the sitting and lying down position than in the standing position, suggested that the greater return of venous blood may possibly act to lengthen the duration of systole in man. Such a lengthening effect of increased venous return on systole was not demonstrated, however, in the experiments of Patterson, Piper and Starling (5) who worked with the heart of a "heart-lung preparation." On the other hand, these investigators observed that an increased arterial resistance consistently lengthens the contraction phase.

In view of the practical bearing that this problem has on the interpretation of many dynamic reactions, and in view also of the apparent discrepancies suggested in the recent work, it seemed appropriate to submit this question to a more fundamental analysis. This could only be accomplished, however, by determining the effects of different procedures on each of the consecutive subdivisions of the cardiac cycle.

METHODS AND PROCEDURES

Since, under physiological as well as pathological and pharmacodynamic conditions, three variables may possibly affect the durations of the consecutive phases of systole and diastole, viz., changes in heart rate, venous return and arterial resistance, experiments were instituted to test the reaction of these factors, when operating singly or combined. In order to do this, intact animals were used exclusively because, on mature reflection, it was felt that the use of a heart-lung preparation introduced fully as many uncontrollable variables as the employment of the intact animals, and would moreover be apt to show what *can* take place rather than what *does occur* in the body.[1]

In order to study the effects produced by variations in heart rate, the vagus nerves were divided or stimulated directly. In order to prevent changes in rate when arterial resistance and venous inflow were altered, it was necessary to divide both vagi nerves. The volume of venous return was reduced by compression of the inferior vena cava

[1] For a full discussion of this problem, consult Wiggers, Harvey Lecture published in the Archives of Internal Medicine 1921, xxvii, 475.

and augmented by a graded inflow of saline solution. In such cases records of the resulting effective venous pressure rather than of the amount injected were kept. Arterial resistance was increased by the production of peripheral vasoconstriction and also by mechanical compression of the abdominal or ascending portions of the aorta. Whenever possible, experiments were carried out both on animals with thorax opened and on those in which the chest remained intact.

THE INFLUENCE OF INCREASING THE VOLUME OF VENOUS RETURN

In interpreting the uncomplicated effect of an augmentation of venous return occasioned by saline infusion, it was important that the venous pressure should not at the outset be below normal. Only those experiments in which an effective right auricular pressure above 55 mm. saline solution existed are therefore included in this analysis. It is also important that concomitant changes in heart rate or arterial resistance should preferably not occur at all or, if taking place, that they be taken into account. While, in many animals, saline infusion caused no appreciable change in heart rate when both vagi had been severed, minor variations frequently occurred. Their possible influence on the duration of systolic phases, as will appear in later discussion, could always be evaluated by bearing in mind that slowing alone has a natural tendency to lengthen systole; acceleration, the reverse effect. Arterial resistance always increased to some extent but, owing to the slow rate of inflow and the reduced viscosity of the blood, systemic diastolic pressure was not markedly altered (cf. table 1). In fact, it was shown in control experiments that a similar degree of resistance increase was either without effect on systole or tended to operate in an opposite manner. It is therefore possible to clearly analyze the specific effects resulting from an increased venous return.

Effect on relative duration of systole and diastole. If, during the course of a venous infusion, the heart rate is counted it is usually found that in spite of vagotomy a slight retardation of the beat takes place, and that this may progress as the infusion continues. Comparison of the periods of systole and diastole show, however, that the slowing is of an entirely different order than when the vagus is stimulated; it is systole rather than diastole that lengthens. The sequence of these changes may be followed by data taken from experiment 231, x, which are incorporated in table 1. The periods obtaining under normal conditions are shown as *a*. Very soon after infusion started, the heart slowed, *b*, due to an increase both in the length of systole and diastole.

TABLE 1

The influence of increased venous return on the duration of consecutive phases of systole and diastole

EXPERIMENT NO.	SYSTOLIC PHASES				DIASTOLIC PHASES					DIASTOLIC ARTERIAL PRESSURE	INITIAL PRESSURE (MM. Hg)
	Isometric contraction	Maximum ejection	Reduced ejection	Total systole	Proto-diastole	Isometric relaxation	Rapid inflow	Diastasis	Total diastole		
231, X											
a	0.055–0.058	0.062–0.080	0.060–0.070	0.185–0.208	0.020–0.030	0.040–0.047	0.047–0.055		0.222–0.234	68	4.2
†b	0.045–0.055	0.070–0.080	0.116–0.125	0.240–0.246	0.020–0.024	0.050–0.058	0.070–0.078	0.024–0.036	0.278–0.284	68	4.8
†c	0.040–0.047	0.068–0.070	0.143–0.155	0.253–0.270	0.020–0.030	0.060–0.070	0.058–0.068		0.230–0.233	70	9.1
†d	0.045	0.074–0.080	0.105–0.112	0.229–0.232	0.020–0.024	0.049–0.058	? –0.055		0.208–0.219	72	7.9
230, XIV											
a	0.052–0.057	0.065–0.070	0.073–0.077	0.190–0.200	0.025–0.032	0.040–0.045	0.032–0.050		0.175–0.192	44	4.8
*a′	0.050–0.052	0.100–0.102	0.070–0.078	0.222–0.223	0.026–0.035	0.040–0.046	0.040–0.048	0.272–0.285	0.470–0.483	33	7.1
†b	0.050–0.053	0.055	0.096–0.097	0.201–0.205	0.028–0.030	0.040–0.044	0.036–0.038		0.198–0.202	43	5.2
†d	0.043–0.050	0.080–0.088	0.09	0.220–0.223	0.03	0.045–0.052	0.040–0.042		0.193–0.197	42	6.9
*†e	0.048–0.053	0.106–0.140	0.098–0.118	0.250–0.295	0.025–0.035	0.050–0.053	0.040–0.06	0.162–0.560	0.303–0.787	33	9.2
†f	0.048–0.050	0.084–0.098	0.100–0.110	0.238–0.252	0.022–0.028	0.045–0.050	0.042–0.052		0.189–0.190	40	7.1
233, X											
a	0.050–0.052	0.063–0.067	0.118–0.120	0.234–0.237	0.020–0.026	0.040–0.048	0.064–0.075	0.155–0.169	0.400–0.412	42	5.3
†b	0.048–0.056	0.07	0.190–0.206	0.308–0.334	0.025–0.030	0.046–0.052	0.048–0.054	0.060–0.720	0.251–0.961	34–42	6.8
†c	0.048–0.054	0.062–0.064	0.140–0.170	0.250–0.280	0.030	0.030	0.048	0.290	0.468	38	8.3
†d	0.044–0.048	0.090–0.093	0.162–0.170	0.292–0.308	0.020–0.022	0.040–0.042	0.040–0.042	?	0.238–0.245	63	?
†e	0.040–0.045	0.090–0.098	0.170–0.172	0.300–0.315	0.028–0.030	0.040	0.045	0.02	0.223–0.228	45	10.3

* Indicates peripheral vagus stimulátion.

† Indicates observations during increased venous return.

In *c*, the duration of diastole returned to normal but systole lengthened still more. After discontinuing infusion, as shown in *d*, diastole became shorter than it was at the start, but systole continued long. If the vagus nerves are stimulated at the time when systole has lengthened independent of diastole, a further lengthening of systole as well as diastole takes place. This is illustrated in experiment 230, xiv, table 1. The data shown as *a* and *a'* indicate control effects of vagus stimulation when conditions were normal just before infusion started. The maintenance of normal diastolic length and the gradual lengthening of systole during infusion are shown by *b* and *d*. Records from which these data were derived were taken early during the infusion process. When the vagus nerve was stimulated in addition and with the same strength of current as had previously been used, a very great slowing accompanied by a further lengthening of systole took place, *e*. After cessation of stimulation, the length of diastole returned to normal but systole remained lengthened, *f*.

Such results illustrate the response of a rapidly beating heart. It still remains to test the reaction of a heart normally beating at a rate which more nearly approximates that in man. Since it was important to avoid the influence of a varying vagal tonus, it was necessary to apply a continued electrical stimulus to the cut vagi and, during the course of such slowing, saline infusion was given. The results of such an experiment are shown in experiment 233, x, table 1. The data listed as *a* show the phasic relations existing during a mild stimulation of the left vagus which, in this case, produced a typical sinus slowing. While stimulation continued, infusion was started and during its continuance observations *b* and *c* were made. The data show without further comment that there is the same tendency for systole to lengthen independently of diastole.

Having shown that the duration of systole may increase independently of diastole when the return flow to the heart is increased, it remains to show that the effects were not primarily due to the increased arterial pressures against which the heart must expel its blood, especially so as Patterson, Piper and Starling found that such an increase of arterial resistance causes a lengthening of the contraction phase. In the first place, as shown by the diastolic pressure readings taken throughout these observations, the administration of saline infusion, as carried out in these experiments, does not cause any appreciable elevation of systemic pressure; in some cases a lengthening of systole occurs without any variation of pressure. When the blood pressure lowers,

as during vagus stimulation (e.g., exper. 230, xiv, *e*), systole lengthens still further and, as the heart subsequently accelerates (e.g., *f*), and before the blood pressure has returned to its former level, the systoles still remain long. It is possible, however, to reduce suddenly the arterial pressure during saline infusion, by opening up a carotid artery, thus allowing a profuse hemorrhage to take place for a short time. Data from such an experiment are illustrated in experiment 233, *e*. They show when compared with normal data, *d*, that systolic length is certainly not reduced, even though diastole may decrease slightly. As will be apparent later, such controls and others that have been employed might have been dispensed with entirely for, contrary to the findings of Patterson, Piper and Starling, an increased arterial resistance of even greater magnitude does not lengthen systole in the intact heart; but, on the contrary, tends to abbreviate it.

Influence on consecutive phases of systole. Tabulations of many experiments, of which those incorporated in table 1 are but specimen summaries, have shown that there is always a tendency for the isometric contraction phase to become abbreviated. Sometimes this abbreviation is marked, again it may be very slight. The following reductions have been noted in different experiments: 0.04 to 0.028; 0.043 to 0.035; 0.05 to 0.032; 0.052 to 0.033; 0.05 to 0.045; 0.055 to 0.052; 0.042 to 0.04; 0.048 to 0.046; 0.055 to 0.04; 0.058 to 0.047; 0.05 to 0.048; 0.051 to 0.056 (experimental increase); 0.052 to 0.048; 0.057 to 0.05. These observations bear out the results of Hürthle (6) and others who found that the duration of this phase is not related to the absolute diastolic pressure in the aorta but is determined rather by the difference between aortic and intraventricular pressures at the beginning of systole. Hürthle inclined to the opinion that the height of the initial pressure within the ventricle at the moment of systole is the fundamental factor. This interpretation is borne out by the results of this research (cf. table 1).

As the isometric contraction phase decreases in length during the continuance of an infusion, the combined phases of ejection lengthen. This indicates that the duration of the ejection process is adapted to the volume of blood which needs to be expelled. A study of the tables reveals no constancy as to whether the phase of maximal or reduced ejection is predominantly affected. As a rule, the latter phase is lengthened first and to greater degree; while the phase of maximal ejection does not increase until a considerable ventricular distention has supervened (cf. table 1). This is, naturally, of no fundamental

significance as bearing upon the nature of the ventricular behavior, but merely means that the flow of diluted blood from the peripheral arterial system keeps pace with the increased ventricular discharge early in systole. This, it may be noted, however, would not be the case if a secondary increase in arterial resistance were in any manner concerned with the increase in length of systole, and strengthens our belief that the rate of venous inflow is the direct factor involved.

There is apparently no doubt that an increased venous return and the consequently greater ventricular filling acts to shorten the isometric contraction phase and to lengthen the ejection phases, thereby prolonging systole as a whole. The question remains whether this is fundamentally due to an increase in diastolic volume (i.e., greater initial length) or to an increase in initial tension. The intraventricular changes occurring in those experiments of this series in which the chest had been opened have recently been reported and analyzed (7). They showed such clear and prompt correspondence between volume and initial pressure alterations that the conclusions seemed necessary that initial pressure changes were fundamentally responsible for such volume changes as occurred. Consequently, the conclusion is favored that the effects which the volume of venous return exerts on the phases of systole are fundamentally related to changes in initial intraventricular pressures.

Influence on consecutive phases of diastole. It is conceivable that the time required for the semilunar valves to close (i.e., the proto-diastolic phase) might be determined by the diastolic pressure existing in the aorta at this time. Apparently this interval is so short, however, and the pressure elevation so insignificant that no striking variations occur (cf. table 1). The data also reveal no marked changes in the duration of the isometric relaxation phase in the majority of cases. Occasionally, as shown in experiment 231, x, this phase lengthens by about 0.02 second. The cause of such an occasional prolongation is not clear. Suffice it to state that the causes which might be logically invoked to explain such a change do not appear to exist during saline infusion. They will be discussed more in detail later (cf. page 450).

Of greater importance as concerning the dynamics of the ventricle are the data relating to the length of the rapid inflow phase. The increased systolic discharge during saline infusion is necessarily due to a greater diastolic inflow. How is this brought about? Is the phase of rapid inflow prolonged, or does the greater filling occur in the same time interval? Tabulated data from these experiments fail to show that this phase is lengthened. It is true that when a rapid heart (in

which the phase of rapid inflow is abridged by a succeeding systole) slows down during the course of an infusion, this phase may be lengthened. This, however, is only a function of heart rate and would have occurred regardless of infusion. In cases in which the heart previous to infusion was sufficiently slow to permit of the existence of a diastasis phase, the phase of rapid inflow was certainly not lengthened (e.g., exper. 233, x, table 1).

THE INFLUENCE OF INCREASED ARTERIAL RESISTANCE

The arterial resistance against which blood must be expelled was augmented in three different ways, viz., *a*, by creating increased resistance in the terminal branches of the arterial tree through reflex vasoconstriction (stimulation of the central vagus nerve); *b*, by compressing the abdominal or lower thoracic aorta; and *c*, by compressing the ascending aorta near the semilunar valves (aortic stenosis). As before, the vagus nerves were cut, except as noted.

Effects on systole and diastole ratios. The net results of these experiments may first be summarized as follows: At constant heart rates, increasing the arterial resistance by vasoconstriction uniformly causes a decrease in the length of systole independent of changes in diastole. Similar changes usually follow compression of the abdominal or lower thoacic aorta. In a few cases the length of systole remains unchanged, however; in a few others it slightly lengthens. The former results, it may be pointed out, are contrary to, the latter in accord with the reactions reported by Patterson, Piper and Starling (5). During aortic stenosis systole invariably lengthens, thus confirming results already reported by de Heer (8).

These effects may be illustrated by reference to a few reactions tabulated in table 2. In experiment 219, ii, the effects of increased resistance following reflex vasoconstriction, *b*, are compared with normal values, *a*. A definite shortening of systole without any alteration in the length of diastole is seen. As shown in experiment 219, vii, *a* and *c*, a similar reduction of systole followed aortic compression even though diastole lengthened somewhat. Experiment 221, iii, shows normal data. After compression of the lower thoracic aorta as shown by data, v, systole decreased in length, diastole remained unchanged. When the aorta was stenosed in its ascending portion, however, as shown in vi, systole, on the contrary, lengthened, while diastole shortened. The data of experiment 231, vii, (table 2) are especially interesting, inasmuch as we have already considered the effects of increased

TABLE 2

The influence of increased arterial resistance on the duration of consecutive phases of the cardiac cycle

EXPERIMENT NO.	SYSTOLIC PHASES				DIASTOLIC PHASES					DIASTOLIC ARTERIAL PRESSURE	INITIAL PRESSURE (MM. Hg)
	Isometric contraction	Maximum ejection	Reduced ejection	Total systole	Proto-diastole	Isometric relaxation	Rapid inflow	Diastasis	Total diastole		
219, II											
a	0.040–0.052	0.065–0.085	0.052–0.068	0.167–0.180	0.015–0.020	0.060–0.085	0.050		0.200–0.230	35	8.0
*b	0.043	0.070	0.032–0.040	0.145–0.153	0.020	0.072–0.088	0.046		0.208–0.229	58	8.5
VII											
a	0.036–0.040	0.060–0.070	0.070	0.170–0.176	0.015–0.020	0.062–0.070	0.050–0.055		0.197–0.215	48	7.0
†c	0.040	0.055	0.065–0.070	0.160–0.165	0.011–0.020	0.091–0.098	0.062		0.223–0.242	63	12.0
221, III	0.058–0.06	0.110–0.115	0.085–0.090	0.258–0.260	0.020–0.022	0.085–0.089	0.060–0.065	0.025–0.030	0.275–0.292	52	7.4
†V	0.032–0.041	0.085–0.090	0.050–0.055	0.172–0.180	0.010–0.014	0.145–0.152	0.06	0.040–0.045	0.282–0.292	79	8.0
††VI	0.032–0.035	0.145–0.150	0.095–0.102	0.275–0.287					0.200–0.215	89	14.8
231, VII											
a	0.052–0.060	0.078–0.080	0.065–0.074	0.202–0.206	0.018–0.020	0.062–0.07	0.064–0.070		0.232–0.240	98	4.8
†b	0.050–0.055	0.067–0.072	0.062–0.070	0.184–0.190	0.013–0.014	0.076	0.055–0.060		0.232–0.234	108	5.3
‡†c	0.056–0.054	0.076–0.078	0.102	0.228–0.234	0.020–0.026	0.075–0.075	0.080–0.095	0.204	0.595–0.625	100	9.7
†d	0.042–0.045	0.100–0.110	0.050–0.055	0.195–0.200	0.016–0.025	0.068–0.073	0.100– (?)		0.246–0.265	138	5.6
XI	0.045–0.046	0.070–0.080	0.068–0.070	0.186–0.193	0.030–0.032	0.065–0.070	?	?	0.245–0.252	44	6.2
††XII	0.065–0.070	0.085–0.098	0.040–0.050	0.200–0.218	0.015–0.020	0.080–0.090	?	?	0.200–0.205	82	11.8
XVIII											
a	0.04	0.070–0.080	0.105–0.110	0.220–0.225	0.025–0.037				0.265–0.295	36	6.0
††b	0.035–0.04	0.150–0.175	0.040–0.045	0.235–0.245	0.020–0.03				0.260–0.275	79	14.2
234, I	0.050–0.060	0.100–0.110	0.09 –0.105	0.253–0.260	0 020–0.022	0.048–0.058	0.055–0.070	0.185–0.255	0.452–0.496	90	9.3
†V											
c	0.049–0.060	0.143–0.178	0.00 –0.040	0.227–0.243	0.020–0.022	0.070–0.080	0.060–0.088	0.232–0.244	0.484–0.548	104	9.7
†d	0.064–0.068	0.126–0.15	0.038–0.04	0.234–0.252	0.022–0.025	0.074–0.083	0.083–0.102	0.335–0.427	0.659–0.735	109	9.9

* Increased vasoconstriction.
† Aortic compression.
†† Aortic stenosis.
‡ Vagus stimulation.

venous inflow in this same experiment (table 1), *a* represents data of normal conditions in the closed chest; *b*, data obtained during compression of the abdominal aorta; a reduction of systole occurred without a change in the duration of diastole; *c* represents results obtained while the peripheral end of the right vagus was stimulated during compression of the aorta; systole lengthened with diastole; *d* shows effects shortly after cessation of stimulation while aortic compression was still retained, increased resistance clearly reduced the periods of systole. The chest was opened and the study continued; xi shows results of control data under these conditions; xii shows a lengthening of systole during moderate aortic stenosis.- Similar lengthening is shown by comparing xviii, *b*, with the normal, *a*. In experiment 234 i represents data under normal conditions; v, *c*, the effect of compressing the abdominal aorta, and v, *d*, the effect of additional slowing. Systole was reduced as compared with normal even though diastole lengthened appreciably. *These data clearly show that increased resistance produced near the semilunar valves uniformly lengthens systole, while if the resistance change occurs more peripherally it tends to abbreviate systole.*

How may these contrary effects be explained? A clue was given by the observations that systole lengthens whenever the initial pressure is greatly elevated but that it shortens whenever initial pressure increases but little. In other words, the fact seems to stand out that *whenever initial pressure in the left ventricle increases considerably, whether as a result of increased resistance to ejection or from increased venous inflow, systole always lengthens.* Increased resistance without a material elevation of initial pressure has the contrary effect of abbreviating systole.

These reactions are in accord with those of skeletal muscles: If the initial tension of an after-loaded skeletal muscle is kept constant, while the lifted load is somewhat increased, the duration of the contraction phase is reduced, for the contractile stress is apparently overpowered sooner by a heavy than by a light load. If, however, the initial tension and initial length are increased at the same time that the lifted load becomes greater, then the contraction phase may lengthen. Returning to these experiments, it is evident that the chief variable introduced in the different experimental methods used to augment resistance consists in the length of elastic arteries interposed between the heart and the area where the resistance was actually increased. When the arterioles contract, diastolic pressure may be markedly elevated but a considerable stretch of elastic artery is still available to accommodate

any blood that may be ejected during systole. Consequently systolic retention is small, diastolic volume not greatly increased and the initial tension in the left ventricle rises but little. In such cases the greater arterial load acts to abbreviate systole. When the abdominal aorta is compressed, the accommodative power of the larger arteries is reduced, and the increase in diastolic volume and initial tension are not enough to overbalance the tendency of increased resistance to shorten systolic ejection. During aortic stenosis, however, the stretch of elastic aorta capable of accommodating ejected blood practically vanishes, consequently a large volume of blood must be retained within the ventricles at the end of each systole. This elevates the initial tension sufficiently to lenthen systole.

Such effects were also obtained by de Heer after production of aortic stenosis and appear to be the only conditions reproduced in the heart-lung preparation by Patterson, Piper and Starling. It is quite conceivable why this was the case. In such a preparation a rubber tube of comparatively small diameter was substituted for the aorta and its branches and all the ejected fluid was required to pass through a narrow cannula tied into the innominate artery. Under such artificial conditions the accommodative capacity of the system reaches its limits readily even when the arterial resistance is increased more peripherally.

Influence on systolic phases. Turning to the effects on the detailed phases of systole, we note no regularity in the changes of the isometric contraction phase. As a rule, the length of this phase varies little when resistance increases due to peripheral constriction or aortic compression, but if anything, a slight tendency to decrease is noted (e.g., exper. 219, ii, *b*, 221, v). During aortic stenosis, on the other hand, the phase generally lengthens somewhat (exper. 231, xii). Exceptions or, better stated, opposite effects occur, however, in many experiments (e.g., exper. 219, vii, 221, vi). The failure of this phase to lengthen consistently in all experiments emphasizes the relatively small importance which aortic pressure plays in determining the length of this phase. The actual shortening is undoubtedly explained as an effect of increased initial tension.

When systole shortens as a result of increased resistance of peripheral origin, the reduction occurs chiefly in the ejection phases. As a rule, the phase of reduced ejection is affected most and in extreme cases may practically disappear (exper. 219, ii). When, during aortic stenosis, systole lengthens, the maximum ejection phase is always prolonged

while the phase of reduced ejection is generally abridged (exper. 231, xii). Occasionally however it too may lengthen (e.g., exper. 221, vi).

Influence on phases of diastole. It may be anticipated that the great pressure existing at the very end of systole may act to close the semilunar valves more rapidly, i.e., decrease the proto-diastolic interval. Such a reduction was found in about 50 per cent of my experiments, being illustrated in table 2, by comparing the data of experiment 221, iii with v; experiment 231, vii, *a* with vii, *b* and experiment 231, xi with xii. The abbreviation, as might have been expected, is the more evident the nearer the aortic valves the resistance change takes place. The duration of the isometric relaxation phase was practically unaltered when increased resistance was occasioned by peripheral constriction (e.g., exper. 219, ii, *a*, *b*). In some experiments in which the abdominal aorta was compressed (e.g., exper. 221, iii, v) and in all cases were aortic stenosis supervened, (e.g., exper. 231, xi, xii) the phase lengthened definitely. While it is difficult to give a logical explanation of these changes, four factors, acting separately or together, may be thought of as effecting an increase in the length of an isometric relaxation phase, viz.:

1. The inherent rate of muscular relaxation during the isometric phase may be slower.

2. Intraventricular pressure being higher at the beginning of this phase, it might at the same rate of relaxation take more time for the pressure to decrease to the level of auricular pressure.

3. Since the opening of the a-v valves is determined by the balance of pressure in the auricle and ventricle toward the end of this phase, it is possible that either an increased intraventricular or a reduced auricular pressure might delay the opening of the a-v valves and hence prolong the isometric relaxation phase.

4. Finally, it is possible that the time interval by which the preceding protodiastolic phase is abridged is added to the phase of isometric relaxation.

After the a-v valves have opened, the continuation of the early diastolic inflow phase depends solely on the length of time during which a pressure difference between auricle and ventricle is maintained. A diastolic retention of blood and an increased intraventricular pressure certainly occur in cases of pronounced aortic stenosis. Consequently, it may be anticipated that the elevation of intraventricular pressure would much sooner retard the inflow and act to reduce the duration of this phase in this case. In none of the experiments in which the heart

was slow enough for a phase of diastasis to exist, was there, however, any evidence that the inflow phase is abridged during augmented arterial resistance of any kind. On the contrary there is, as shown in experiment 231, vii, *c*, at times a slight tendency for this phase to lengthen.

THE INFLUENCE OF HEART RATE

Influence on duration of systole and diastole. Section of both vagi nerves producing a tachycardia by giving the tonically active accelerators unopposed control, uniformly decreases the duration of systole even when, as happened a few times, the length of diastole is unaffected. The injection of small doses of epinephrin causes a further decrease in the length of systole which persists even when the vagi are stimulated in addition, and the heart in consequence becomes very slow.

The details of an experiment substantiating these deductions are summarized in table 3, experiment 231.

The changes that take place when the heart is slowed depend on the method through which the inhibitory influence of the vagus is brought into action. When the peripheral end of a vagus nerve is stimulated electrically, the systole: diastole ratios conform to a standard curve only when new and constant dynamic relations have been reëstablished (e.g., exper. 228, viii). During the time that the systemic pressures decline, changes in systolic length are in no sense related to the length of previous diastole. There is constantly a progressive increase in the duration of systole as stimulation continues and this occurs quite regardless of previous diastole. When stimulation has ceased, the diastoles are promptly shortened but systoles only gradually and progressively regain their former lengths. These observations, illustrated by the plots of experiments 216 and 232 in figure 1, are but confirmatory of independent observations recently published by Katz (3). Such results really need no additional comment, yet attention may be drawn to a striking relation shown in experiment 216. The heart beat at the end of stimulation had a diastolic length of 0.237 second with a systolic period of 0.195 second—this period being precisely equivalent to a systolic interval accompanying an earlier vagal beat having a diastole length of 1.819 second. A similar lack of relation was obtained when the heart was reflexly slowed by stimulation of the central end of one vagus nerve. The significant observation may be added that it often happened that, when conditions had been stabilized, systole was more prolonged by reflex than by direct vagal slowing although

TABLE 3

The effects of stabilized changes in heart-rate on the duration of the consecutive phases of the cardiac cycle

EXPERIMENT NO.	NATURE OF EXPERIMENT	PHASES OF SYSTOLE				PHASES OF DIASTOLE					ARTERIAL PRESSURE	EFFECTIVE VENOUS PRESS. (MM. Hg)
		Isometric contraction	Maximum ejection	Reduced ejection	Total systole	Proto-diastole	Isometric relaxation	Rapid inflow	Diastasis	Total diastole		
228												
I	Control	0.044–0.048	0.080–0.082	0.075–0.080	0.200–0.207	0.021–0.023	0.065–0.078	0.065–0.078	0.04 –0.065	0.291–0.408	74	6.1
IV	Epinephrin	0.020–0.030	0.070–0.085	0.055–0.072	0.159–0.180	0.015–0.022				0.475–1.287	118	7.8
V	Left vagus cut	0.042–0.045	0.075–0.092	0.040–0.060	0.174–0.180	0.015–0.024				0.289–0.299	77	5.8
VI	Central vagus stimulated	0.056–0.055	0.118–0.135	0.082–0.090	0.263–0.267	0.025–0.027				0.657–0.665	98	8.2
VIII	Peripheral vagus stimlated	0.048–0.05	0.110–0.118	0.088–0.098	0.236–0.252	0.024–0.028				0.669–1.198	46	7.4
IX	Normal control	0.045–0.050	0.098–0.102	0.030–0.040	0.177–0.186	0.016–0.020				0.230–0.240	78	6.0
231												
I	Control	0.052–0.058	0.080–0.096	0.058–0.078	0.199–0.216	0.015–0.022	0.040–0.052	0.044–0.055	0.000–0.055	0.250–0.260	94	6.8
II	Right vagus cut	0.048–0.052	0.080–0.086	0.054–0.060	0.183–0.192	0.016–0.025	0.047–0.052	0.044–0.064		0.222–0.234	102	6.4
V	Left vagus cut	0.052–0.058	0.072–0.082	0.064–0.070	0.194–0.210	0.015–0.024	0.042–0.064	0.060–0.068	0.02 –0.03	0.245–0.259	102	6.4

IX												
a	Control later	0.044–0.045	0.078–0.080	0.062–0.068	0.187–0.190	0.018–0.020	0.053–0.056	0.062–0.063		0.235–0.239	100	6.0
b	Epinephrin	0.041–0.045	0.052–0.058	0.038–0.048	0.135–0.143	0.015–p.02	0.052–0.055	0.05		0.185–0.203	132	6.5
c	Peripheral vagus stimulated during epinephrin	0.038–0.045	0.055–0.070	0.038–0.070	0.133–0.182	0.024–0.038	0.045–0.072	0.06 –0.08	0.260–0.330	0.530–0.757	100	7.8
225												
II b	Asphyxia	0.038–0.040	0.098–0.106	0.064–0.095	0.208–0.213	0.018–0.027	0.068–0.073	0.124-0.125	0.160–0.349	0.413–0.702	74	11.3
IV b	Control	0.046–0.056	0.051–0.060	0.072–0.084	0.182–0.186	0.03 –0.033	0.058–0.063	0.080–0.081	0.000	0.218–0.225	51	4.3
219												
a	Control	0.040–0.042	0.073–0.080	0.032–0.038	0.153	0.015	0.063–0.070			0.195	38	7.8
b	Early stage asphyxia	0.040–0.041	0.062–0.065	0.031–0.045	0.147–0.148	0.010–0.014	0.060–0.072			0.184–0.208	59	9.7
c	Later stage	0.035–0.042	0.070	0.022–0.025	0.127–0.134	0.010–0.015	0.058–0.062			0.422–0.485	73	12.8

diastole was always much larger in the latter case (cf. exper. 228, vi and viii, table 3). The effect of vagal slowing by partial asphyxiation was determined in 14 experiments. In 4 cases only was there a lengthening of systole during beats with long diastoles which are at all comparable to direct or reflex vagal stimulation. In two experiments, systole lengthened very little; in two others, no change of systole was apparent although diastole lengthened markedly. Of even greater interest are six other experiments in which systole consistently *decreased* in length while diastole increased. Thus in one of these experiments, the systole : diastole ratio was $\frac{0.175}{0.187}$ before slowing, $\frac{0.149}{0.42}$ and $\frac{0.142}{0.70}$ at different times during slowing process. The changes

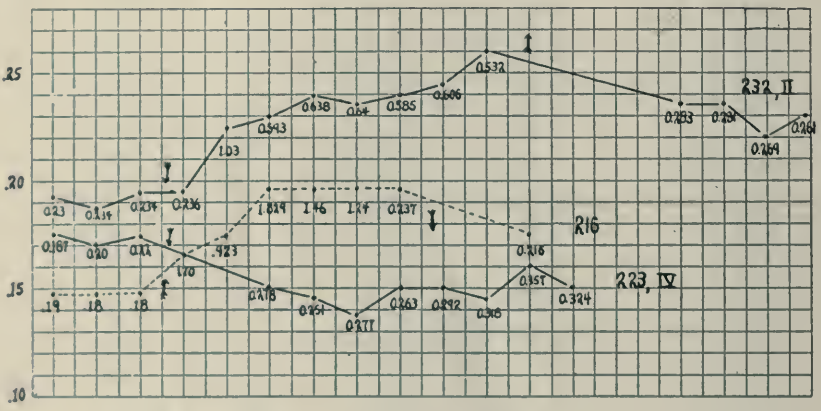

Fig. 1. Plots showing the lack of relation between durations of systoles and preceding diastoles. Experiments 216 and 232, ii, show changes during electrical vagus stimulation; experiment 233, iv, shows shortening effect of asphyxial slowing. Dots on ordinates indicate durations of successive systoles; figures beneath indicate lengths of preceding diastoles.

in consecutive beats of another experiment where only a moderate slowing occurred are shown as plot of experiment 223, in figure 1. Similar reactions take place after the administration of epinephrin. If the vagi are intact and the heart slows, the lengthening of diastole is accompanied by a decrease in the duration of systole (e.g., exper. 228, iv, table 3). If the vagi are divided and one of the vagus nerves is then stimulated while epinephrin is acting, the phase of systole remains diminished while diastole increases in length (e.g., exper. 231, ix, *a*, *b*, *c*, table 3).

It is quite apparent from such observations that the variations of systole when vagal slowing is accompanied by marked dynamic changes do not correspond with those obtained when the vagus is stimulated directly. This is probably accounted for by the fact that still other factors (e.g., simultaneous stimulation of the accelerator nerves, mechanical influence of high blood pressure or altered venous return) affect the duration of systole in an opposite direction. The balance of such opposing forces determines whether systole actually lengthens, shortens or remains unaltered as the cycles change. The detailed factors at work will be analyzed more at length in a subsequent portion of this paper. It may be profitable, however, at once to direct attention to the fact that high arterial pressure is one influence capable of counteracting the lengthening influence exerted by vagus slowing. Thus, in experiment 234, i, *v*, *c* (table 2) in which both vagi nerves were left intact, compression of the abdominal aorta first decreased the duration of systole without affecting the diastolic length appreciably. When later (*v*, *d*) a natural reflex slowing was initiated by the high arterial pressure, systole lengthened again but not above the duration it had at much more rapid rates.

Effects on systolic phases. As long as marked variations in systemic resistance or initial tension do not occur, the duration of the isometric phase is not permanently altered. This is true, e.g., during cardiac acceleration following vagotomy (e.g., exper. 231, i, v). Similarly there is no permanent change when the peripheral end of the vagus is stimulated (e.g., exper. 231, ix, *c*, 228, viii). When cardiac slowing is accompanied by drastic elevation in arterial resistance and initial tension, e.g., during asphyxia (exper. 225, ii, *b*) or during the action of epinephrin in animals with vagi intact (exper. 228, iv, table 3), then the isometric phase is definitely and permanently reduced. When, on the other hand, epinephrin simultaneously causes a great elevation of arterial pressure and cardiac acceleration in animals with vagi cut, this phase is not affected (exper. 231, ix, *a* and *b*, table 3). This would indicate that the reduction in the isometric phase is attributable neither to a specific effect of epinephrin on the accelerator mechanisms or on the heart itself nor to the height of arterial tension, but rather to the initial tension which determined the rate of pressure development. When epinephrin causes cardiac slowing, initial tension is markedly increased (exper. 228, iv). In cases where it causes acceleration, it is not greatly affected (exper. 231, ix, *b*). That this is indeed a factor is shown in experiment 231, ix, *c* where, upon additional stimulation of

the vagus nerve, the phase is promptly abridged when the initial tension is increased.

Since the isometric contraction phase alters only in exceptional cases, i.e., where the initial tension is greatly increased, and in these, in fact, tends to shorten, it is obvious that alterations in the duration of systole are almost entirely due to changes in the ejection phase. It will be recalled that whenever the heart is slowed through direct or reflex vagus stimulation, the interval of systole progressively increases, and that this is related neither to the duration of previous diastole nor to the diastolic arterial pressure. On the other hand epinephrin,

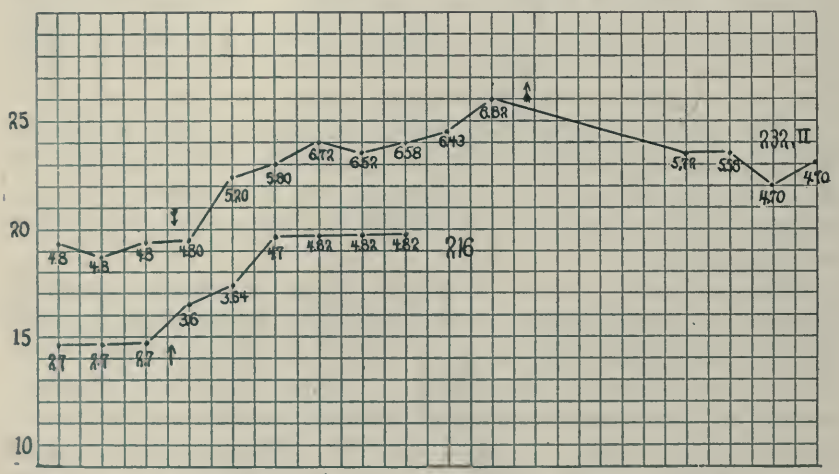

Fig. 2. Plots showing the relation between initial intraventricular pressure and duration of systoles, during vagus slowing. Same experiments and plot as in figure 1, except that figures beneath dots indicate the initial intraventricular pressures in mm. of Hg.

asphyxia or slowing fail to increase this interval. These variations in systolic lengths are paralleled by changes in the length of the ejection phases.

The progressive lengthening of the systolic ejection phases is accompanied by a progressive increase in the initial pressure and in fact no further lengthening occurs when the initial pressure also fails to increase further (fig. 2). Since an increase in initial tension lengthens the ejection phase it is probable that it is the factor which influences the duration of this phase in cases where the heart rate changes. This explains why systolic ejection remains lengthened for a considerable number of beats after the return of the heart to a more rapid rate.

The conclusion may be drawn that when the heart changes its rate, the duration of systolic ejection and hence that of systole is determined not only by the previous diastole length, but often, to a far greater extent, by the variation in initial tension within the ventricle.

It remains to interpret the cases where the systolic ejection was reduced or unaltered, for both during asphyxia and during the action of epinephrin the initial tension is also increased. One and possibly two other factors then counteract such an effect of augmented initial tension to lengthen the period of systolic ejection and consequently also that of systole. It has already been pointed out that a great increase in arterial resistance tends to reduce the ejection phase and, as this operates intensely during asphyxia and epinephrin action, we have two counteracting tendencies at work—the augmented initial pressure tendency to lengthen and the increased arterial resistance acting to abbreviate this phase. The relative influence of these factors determines whether systolic ejection is slightly lengthened, unaltered or, as frequently happens, actually abbreviated.

In the case of slowing induced by epinephrin, at least another factor can not be ruled out, viz., a specific effect on the accelerator mechanisms. That this is concerned is indicated by such observations as are recorded in experiment 231, ix, table 3. In this case a small dose of such a solution caused an increase in the cardiac rate, an elevation of diastolic arterial pressure and a pronounced decrease in the systolic ejection phase. During additional vagus stimulation (ix, *c*) when the arterial pressure returned to normal and in spite of an elevation of initial pressure and a prolonged diastole, systole was still less than normal.

Effects on diastolic phases. Changes in heart rate produce only very minor changes in the duration of the diastolic phases. As before indicated, the duration of the proto-diastolic phase is so short that no pronounced changes may be anticipated. This was the case except in a few experiments in which the duration slightly decreased after direct or reflex vagus stimulation (exper. 228, vi, viii). The duration of the isometric relaxation phase was not affected during slowing beyond the range of error with the exception of two cases (e.g., exper. 231, ix, *c*) in which an increase of about 0.2 second occurred in some beats. Similarly, a slight increase was obtained in three asphyxial experiments. It is apparent that, with few exceptions, which, as a matter of fact, do not show any extreme change in the isometric phase of relaxation, it remains unaltered during various types of vagal slowing. No evidence

has been found that the early diastolic inflow phase increases in length during cardiac slowing of any type (table 3). These observations give probability to the view that, as regards time relations at least, the diastolic inflow period is entirely superimposable during vagus slowing.

SUMMARY AND CONCLUSIONS

Since previous observations had indicated that the duration of systole is not entirely determined by the length of previous diastole, an investigation was undertaken to determine what other factors might be concerned. The following conclusions were reached:

1. An increase in venous return above normal causes a lengthening of ventricular systole, quite independent of diastole length. This occurs as a result of the prolongation of the ejection phases, the isometric phase of contraction having a tendency to be abridged. Since the initial pressure was always elevated when this occurred, we may formulate the conception that, whether or not the mechanical energy liberated by contraction is fundamentally determined by the initial length of the ventricular fibers at the inception of contraction, the duration of systole, at constant heart rates, varies directly as the initial intraventricular pressure under such a variety of inflow conditions as it is possible to produce in the mammalian heart in situ.

2. An increase in arterial resistance acts to abbreviate systole, independently of changes in diastolic length. This abbreviation of systole takes place in spite of a slight tendency of the isometric phase to lengthen and is therefore due to a shortening of the ejection phase. This shortening of the contraction phase is apparently due as in skeletal muscle, to the fact that the muscular shortening is terminated earlier by the greater load acting during the process of shortening.

3. When the increase in arterial resistance is of such grade and type that the ventricle can not empty itself effectively, a marked retention of blood occurs, and the diastolic volume and initial tension increase, in consequence. As such an increase in initial pressure has a tendency to lengthen systole, two opposing influences come into operation in such elevation of arterial resistance, viz., *a*, the tendency of higher arterial resistance to abbreviate; and *b*, the tendency of increased initial tension to lengthen systole. Both factors, no doubt, operate to influence the duration of systole whenever the arterial resistance is increased, but one factor usually predominates. When arterial resistance increases as a result of intense peripheral vasoconstriction, systolic retention is not pronounced and initial tension only slightly

increased. Consequently the first factor predominates, and systole decreases. When the aorta is stenosed, the accommodative capacity of the aorta is reduced and large volumes of blood are retained within the ventricle, consequently the initial tension increases very markedly. The second factor then predominates and systole lengthens. When the elasticity of the arterial system or the caliber of the vessels is insufficient to accommodate readily the systolic volume expelled by the ventricles, an increase in peripheral resistance may also act to elevate initial tension and lengthen systole. This explains the lengthening of systole consistently found by Patterson, Piper and Starling in the heart-lung preparation and we may expect to find such lengthening also in arteriosclerosis involving the larger vessels.

4. The duration of systole is determined solely by changes in the length of previous diastole *only* when the initial intraventricular pressures and the aortic pressure at the beginning of diastole remain nearly constant. Thus we find that, during vagal slowing and during the acceleration subsequent to such stimulation, the duration of systole is predominantly determined by changes in initial pressure, rather than the length of preceding diastole. Again, when slowing occurs in association with an elevation of arterial resistance as during asphyxia or epinephrin, the duration of systole may be abbreviated through the influence of such higher resistance. There is evidence also that in the case of adrenalin action or stimulation of the accelerator nerves, there is a specific effect on the ventricle which acts independent of these influences to shorten the contraction phase.

5. These observations indicate that not only under abnormal conditions of venous and arterial pressures but also under such conditions as may be considered quite normal, the *systolic portion* of the ventricular volume curves can not be regarded as superimposable at different rates of beat.

6. Since the phases of diastole alter so little in duration, however, during the most diverse conditions of the circulation, it is not possible from these observations to draw the same deductions in regard to the superimposability of diastolic portions of the volume curves.

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THE EFFECT OF SHORT SPELLS OF REST ON PHYSICAL EFFICIENCY AS MEASURED BY A BICYCLE ERGOMETER[1]

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Received for publication April 2, 1921

Introduction. It has been stated (1) that when physical labor is in character very severe, an increase in efficiency can be brought about by interrupting the work at rather short intervals with spells of rest. For example, in trench digging the interruption of work every 5 minutes by a period of 10 minutes' rest was found more productive than when the work was performed continuously. Again in the heavy work of moulding in a large munition factory, it was found advantageous from the point of view of production to rest 15 minutes once in every hour.

Wishing to observe a little more minutely the relation of the severity of the exertion to the effect of spells of rest upon production, the following investigation was undertaken.

Method. The method employed was to determine the number of revolutions of the wheel of a bicycle ergometer. Upon this wheel rested a brake which could be weighted to a known degree. Two subjects were used: M. M. J., female, 22 years, physical education student; and P. M. D., male, 46 years, university professor. Each of these subjects performed two series of experiments. The first consisted in riding *continuously* for one hour; the second, in *interrupting* the rides in such a manner that every 10 minutes of riding was followed by a 5-minute period of rest.

Results. The results of these experiments are shown in tables 1 and 2 and figures 1 and 2, which are sufficiently explained in their legends and require only a brief comment in this place.

[1] The material used in writing this article was first collected in the B. A. Thesis of Lucy A. Wallrich submitted to the University of Wisconsin in 1919. It was also reported briefly before the American Physiological Society (this Journal, 1921, lv, 314).

On perusing the tables and figures it is interesting to observe how there is gradually approached and finally passed over a neutral point, a point where it is quite indifferent whether the ride is interrupted by spells of rest or is not. The subject is quite unconscious of this neutral

TABLE 1

KILOS	INTERMITTENT		CONTINUOUS	
	Date	Revolutions	Revolutions	Date
8	January 13	7,810	13,423	January 29
8	January 25	9,391	12,534	February 5
8	February 1	10,312	8,313	February 15
8	February 8	10,160		
8	February 19	9,074		
	Average	9,349	11,423	Average
16	March 7	3,946	2,050	February 27
16	March 25	4,918	3,668	March 22
16	March 28	4,533	3,823	March 31
16	April 2	4,752	3,943	April 4
16	April 5	4,000	4,078	April 6
16	April 11	5,176	6,895	April 9
16	April 21	5,204	6,405	April 14
16	April 26	4,636	6,539	April 18
	Average	4,652	4,550	Average
12	April 28	6,370	7,740	May 6

Table 1. Shows the results of the two series of rides performed by the subject P. M. D. Note *a*, with 8 kilo brake the number of revolutions is usually higher (average much higher) when the ride is continuous; *b*, with 12 kilos only two rides were performed; of these the continuous ride showed a greater number of revolutions; *c*, with 16 kilo brake the number of revolutions was at first much less when the ride was continuous and then much greater. The averages in this case are not significant as it is certain that the relation would have been reversed had the number of rides been increased.

point and of his relation to it. Doubtless the passage over this point in the reverse direction occurs during relatively slight indispositions. The necessity of fitting the task not only to the particular operative or athlete but to the particular physical condition in which the individual finds himself is obvious.

TABLE 2

KILOS	INTERMITTENT		CONTINUOUS	
	Date	Revolutions	Revolutions	Date
4	January 18	4,933		
4	January 22	5,105		
4	January 24	5,659		
4	January 25	5,577		
4	January 29	5,906		
4	January 31	6,091		
4	February 1	6,412		
4	February 6	5,294		
4	February 7	5,438		
4	February 19	6,783	10,233	February 20
4	February 26	5,910	10,745	February 24
	Average	5,737	10,489	Average
8	March 7	5,790		
8	March 8	5,402		
8	April 1	5,896	7,361	April 2
8	April 3	5,945	6,620	April 4
	Average	5,758	6,990	Average
12	April 8	4,039	4,890	April 17
12	April 19	4,444	5,152	May 13
12	May 7	4,359	5,948	May 10
	Average	4,281	5,330	Average

Table 2. Shows the results of the two series of rides performed by the subject M. M. J. Note *a*, with 4 kilo brake the number is always much higher when the ride is continuous; *b*, with 8 kilos the same is true but the difference between the results of the continuous and those of the intermittent rides is less; *c*, with 12 kilos the difference is still less although it remains in favor of the continuous rides. Without doubt the addition of a couple more kilos would—as in the other subject (P. M. D.)—have transferred the advantage to the intermittent ride.

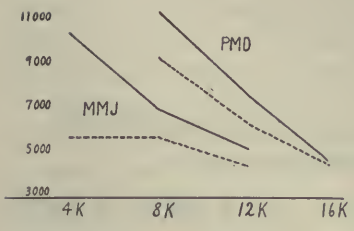

Fig. 1

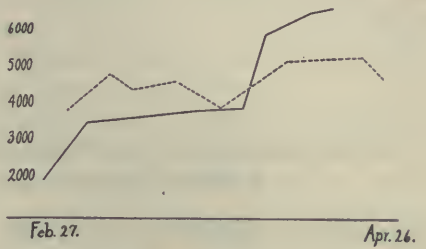

Fig. 2

Fig. 1. Effect of increasing the weight upon the number of revolutions per hour. Ordinates represent number of revolutions of cycle ergometer; abscissae, weight on brake; solid lines, continuous riding; broken lines, interrupted riding. Each point on the curves represents the average of several rides. *Note that as the weight upon the brake was increased the advantage of the continuous over the interrupted work was decreased.*

Fig. 2. Effect of practice and improved physical condition upon the number of revolutions per hour with a heavy brake; 16 kilos. Ordinates represent number of revolutions of cycle ergometer; abscissae sequence of rides from February 27 to April 26; solid lines, continuous riding; broken lines, interrupted riding. Each point on the curves represents a single ride. *Note that as the subject became practiced and his physical condition improved, the advantage of the spells of rest decreased, disappeared and was finally transformed into disadvantage.*

CONCLUSIONS

1. When exercise is *heavy*, efficiency is increased by spells of rest.
2. When exercise is *light*, efficiency is decreased by spells of rest.
3. The neutral point, viz., where work is neither light nor heavy, varies with the individual.
4. The neutral point varies in the same individual depending on practice and physical condition (training).

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THE SURFACE TEMPERATURE OF THE ELEPHANT, RHINOCEROS AND HIPPOPOTAMUS

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Received for publication April 4, 1921

The importance in the animal economy of the loss of heat by radiation, conduction and vaporization of water, justifies a study of the factors governing this loss. Among these factors of no small significance is the surface, i.e., skin temperature, particularly as influenced by the nature of the integument and by the presence or absence of protective covering, such as hair, fur, feathers or, in the case of civilized man, clothing. Observations of the skin temperature of both clothed and nude humans have been in progress in the Nutrition Laboratory for a number of years. With many of the domestic animals numerous data for the skin temperature are recorded in the literature, but these were for the most part taken outside of the fur covering or the attempt was made to secure the observations at the skin well covered with fur. It is obvious that skin temperature measurements obtained under these conditions are not only uncertain but likewise difficult of interpretation, because of the large mass of stagnant warm air contained in the fur. For comparison with the data obtained with nude humans, therefore, the most accurate and most immediate results are secured only with those lower animals that are hairless.

In connection with a series of observations at the New York Zoölogical Park, which has coöperated with the Nutrition Laboratory for a number of years in the study of the metabolism of some of the lower animals, it became possible to obtain skin temperature measurements on several of the large pachyderms. The liberal scientific policy of Dr. W. T. Hornaday, the Director of the New York Zoölogical Park, and particularly the friendly coöperation of Mr. Raymond L. Ditmars, the Assistant Curator of Mammals and Curator of Reptiles, enabled us to make an intensive study of the skin temperature of two elephants, one rhinoceros and a hippopotamus. These animals are, for the most part,

well used to handling by man, and while obviously in captivity and therefore under artificial conditions, nevertheless have lived under these conditions for a decade or more and hence may be fairly said to have adjusted themselves to their present environment.

A thermo-electric method of taking skin temperature measurements has been carefully tested in the Nutrition Laboratory and a preliminary communication has already been made with regard to certain results obtained upon a human subject, both clothed and nude, after several hours of exposure to cold.[1] Since the animals chosen for the study reported in this second paper were in many instances very restless and only a few seconds could at times be taken to secure the temperature records, this thermo-electric method was ideally adapted for our purpose as it permits extremely rapid and accurate measurements. Fortunately, repeated tests of our apparatus have shown that a 6-second period of application of the thermo-junction suffices to give the true skin temperature.

The apparatus consists of two copper-constantan thermo-junctions, one of which is applied directly to the skin, while the other is placed in a thermos bottle carrying a tenth degree Centigrade thermometer, which can be read to hundredths. The terminal wires lead to a galvanometer which is of such a degree of sensitivity that each millimeter scale deflection corresponds approximately to 0.06°C. The galvanometer was of a fairly low resistance, long leads could easily be used, and this made it possible to go into the farther corners of the several animal compartments without having to carry in delicate electrical apparatus, with the danger of breakage. The constant temperature junction (held at about 30°C.) was read practically every 3 or 4 minutes, and approximately every hour the galvanometer "constant" was verified. In the operation of taking the temperature measurements one of us was at the galvanometer to read and make records of the deflections on the scale, one applied the junction to the skin, and the third determined with a pen point on a series of sketches, outline drawings or picture postcards, the exact location of the measurement, this location being given a number for subsequent reference. With a piece of chalk each point of application was marked on the animal's hide, and subsequent readings could be taken at the same point for a check and control.

The skin temperature of a female Indian elephant and a male African elephant. The first animal studied was a female Indian elephant, *Elephas indicus* (*Cuv.*). At the time of our measurements she was

[1] Benedict, Miles and Johnson: Proc. Nat. Acad. Sci., 1919, v, 218.

25 years old, her estimated weight was 6300 pounds, and she was 8 feet $5\frac{1}{2}$ inches tall, 10 feet $7\frac{3}{4}$ inches long, and 4 feet 1 inch broad. This animal, while thoroughly tractable and frequently used in summer to carry children about the Park, was very nervous. The application of the thermo-junction seemed to startle her and it required some training to secure satisfactory readings. The incessant activity of the animal likewise made the measurements difficult. Indeed, it is probably correct to state that at no time during our entire experience, covering several days, was either elephant motionless for two consecutive seconds.

While the temperature of the elephant house was kept relatively constant at about 19.5°C., the weather at the time of our observations was extremely cold and not infrequently a perceptibly cold draft from one of the skylights and pronounced wind drafts from the wall could be felt about the elephant's body. We have reason to believe that these drafts at times materially affected the surface temperatures of the animals. Thus, for example, when the series of measurements was repeated on the second day, considerable differences in skin temperature amounting to 3° or 4°C. were noticed in the same spot. Owing to the inquisitiveness and restlessness of the elephant, it was impossible to place a psychrometer within reach of or anywhere near the animal. The psychrometer was, however, placed around the corner in an adjoining compartment, under practically the same atmospheric conditions, and the temperature of the wet bulb was found to be on the average 10.9°C. throughout our observations.

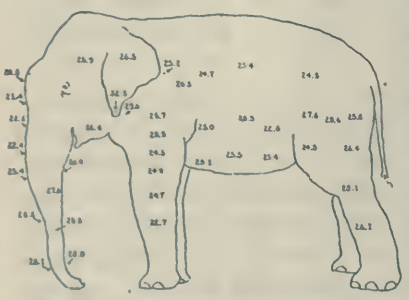

Fig. 1. Surface temperatures of a female Indian elephant (left side). Temperature of environment, 19.5°C.

A topographical survey of the skin temperature of this female Indian elephant is given in figure 1. In this and the following diagrams are given *only* measurements on the unprotected skin areas. Each record represents in most cases the average of a number of measurements, but occasionally a single measurement. The decimal point in each number serves to indicate the exact location of the measurement; in a few instances where this method could not be advantageously adhered to, an arrow points to the location of the measurement. Figure 1 represents the left side of the Indian elephant, since practically all

of the observations were made upon this side. A few temperature measurements were also obtained on the right side of the body, two points on the exterior surface of the ear giving 23.7°C. and 24.1°C., respectively, and two points on the right shoulder giving 26.2°C. and 25.0°C.

The relatively high values found around the lower end of the trunk, particularly on the inside of the trunk, may be explained by the fact that the expired air was constantly being blown against these parts. In the semi-enclosed places, such as the groin and axilla, high values were normally to be expected.

On the ear extraordinarily high temperatures were found. In general, the temperature on the inside of the ear was somewhat warmer than that on the outside, but one would normally expect that the tip end of the ear would be relatively cold, as is the case with humans. As a matter of fact, the warmest spot on this animal's entire surface was on the tip end of the left ear, where two records averaging 32.5°C. were obtained. Although it was impossible to make a very close examination of the ear and the skin was so rough that veins or arteries could not be seen, every effort was made to take a sufficient number of observations to be sure we were not dealing with the temperature taken directly over an artery. The two figures obtained at the tip end of the left ear of 31.6°C. and 33.4°C. may therefore be considered as certainly characteristic of the temperature at this particular point and at the particular moment. Indeed, the temperature at this point was so high that, when called to the attention of the keepers, it was noted by the hand alone.

The right ear of this elephant gave at no point temperatures as high as those found on the left ear, the highest record obtained at any time being 24.1°C. at a point approximately equivalent to where an average of 32.5°C. was found on the left ear. This difference of substantially 8° we are unable to explain. The keepers looked for a possible abscess as a result of the use of an elephant hook, but nothing could be found to account for the high temperature. With the other elephant a similar observation of high temperature on the tip of the ear was made. Furthermore, it should be stated that on the three days during which we studied the elephants, marked differences in the temperature of the two ears and, indeed, very wide and relatively rapid changes in temperature on the periphery of the ear were frequently noted, thus suggesting a large blood supply even to the tip of the ear.

In general, as seen from figure 1, there is a fairly uniform temperature distribution over the skin of this Indian elephant, the average skin temperature being close to 25.5°C.

Skin temperature measurements were likewise secured with a male African elephant, *Elephas oxytis* (*Matschie*), which at the time of our observations was 15 years old, 9 feet $3\frac{1}{4}$ inches tall, 11 feet $1\frac{1}{4}$ inches long, 3 feet 7 inches broad, and had an estimated weight of 6000 pounds. This animal, though not considered so peaceful and tractable as the female Indian elephant, having at times shown a rather unruly disposition, proved to be extraordinarily docile in the hands of his keeper, Mr. Walter Thuman, and, aside from the inevitable inquisitiveness and restlessness, permitted measurements ad libitum.

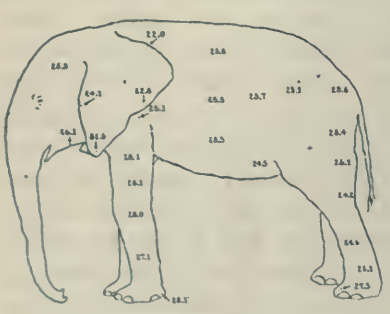

Fig. 2

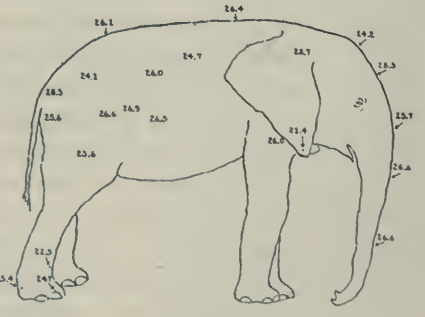

Fig. 3

Fig. 2. Surface temperatures of a male African elephant (left side). Temperature of environment, 19.5°C.

Fig. 3. Surface temperatures of a male African elephant (right side). Temperature of environment, 19.5°C.

Two somewhat extensive series of measurements were made upon him on consecutive days. Both the left and the right sides were studied, primarily with a view to noting if there was anything in the nature of a general unilateral temperature distribution, as suggested by the curious changes noted in the ear temperatures. The average values obtained on the left side of this elephant are charted in figure 2 and those on the right side in figure 3. While a careful study of the figures will show that there was not perfect equality in the temperatures at any two corresponding spots on opposite sides of the body, nevertheless if we take into consideration the differences in temperature of the same spot noted at different times of the day and on different days, there is nothing in the data to warrant even a suspicion of a unilateral temperature

distribution. Attention should be called again to the extraordinarily high values found at the tip of the left ear, where one would ordinarily expect low temperatures. The back of the ear, i.e., in a protected area, had on the whole a higher temperature than the front, and very marked differences were observed in relatively small areas. In this connection it should be noted that the ears of the African elephant are considerably larger than those of the Indian elephant.

The semi-enclosed parts, such as the groin and the axilla, and particularly around the ear and the inside of the trunk, were characterized by relatively high temperatures. The highest individual reading noted on this animal was 33.6°C. well up in the groin. On the soles of the feet temperatures were recorded of 24.3°C. on the left hind foot and 25.4°C. on the left front foot.

All the observations taken with this animal seem to indicate a considerably higher temperature down the front leg than down the hind legs. It should be pointed out that this conclusion is based upon only one series of temperature measurements down the front leg from the shoulders to the toes, and it is to be regretted that a second series was not obtained over this same area on the other front leg. As a matter of fact, the computations of the temperature measurements were not made until some time after the actual observations were recorded, and hence this point was not clearly brought out until later. Since this particular elephant was somewhat sensitive to being touched toward the outer end of the trunk, very few observations could be obtained there. The general picture of the data recorded for this male African elephant shows an average skin temperature essentially that observed with the female Indian elephant, i.e., 25.5°C.

The rectal temperature of elephants has not been extensively recorded. Dr. W. R. Blair of the New York Zoölogical Park has furnished us with the following information with regard to rectal temperatures taken by himself to establish normal values. With the Indian elephant he has found rectal temperatures of 97.4°F., 97.2°F. and 98°F. (i.e., 36.2° to 36.7°C.). Our own observations on the rectal temperature of the African elephant gave values of 35.90°C. and 35.85°C. Realizing that the relatively large masses of feces passed by these animals, falling upon a fairly warm concrete floor, would probably lose heat very slowly in the center, a thermometer was thrust into the warm, moist mass of feces as quickly as it was passed, and a record was thus obtained of 36.7°C. for the temperature of the feces of the female elephant and 36.2°C. for those of the male elephant. It is probably true, therefore,

that the rectal temperature of the elephant is not far from that of the human body, possibly slightly lower than the average usually accepted for man, which is 37°C.

The skin temperature of a black African rhinoceros. The rhinoceros in the New York Zoölogical Park, a female, although reputed to be rather intractable, was, as a matter of fact, a most willing subject. As a rule she remained standing very quietly, and on one occasion actually went to sleep, lying on her side, thus affording the best possible opportunity for obtaining the temperatures in the groin and on the soles of the feet. This animal, of the species known as *Rhinoceros bicornis* (*Linn.*), was born in 1905 and accordingly was 15 years old at the time of our measurements. Her estimated weight was 1900 pounds, and she was 4 feet 8 inches tall, 9 feet 6 inches long, and 2 feet 10 inches broad. Her skin, although extraordinarily thick, was much smoother than that of the elephants and thus gave much more satisfactory measurements.

In figure 4 are recorded only those temperatures found on the exposed surface of the skin on the left side of the body. Obviously in the groin and between the folds of skin high temperatures were noted, but these are not given in this diagram. There is a slight tendency for the lower temperatures to be found along the back rather than farther down the sides and belly. The highest temperature recorded on the skin was 27.9°C. on the left flank. Since the temperature of the environment in the rhinoceros compartment was essentially the same as that in the elephant compartment, it is clear that the rhinoceros had a slightly higher skin temperature than the elephant. In addition to the data given in figure 4 a number of records were obtained in the folds of the heavy skin. One record in a fold at the top of the head directly back of the ear showed 29.2°C. In the left axilla, deep in the fold, a temperature of 29.6°C. was found, while well up in the groin two records of 33.4°C. and 33.3°C. were obtained. Two records on the soles of the front feet gave 28.6°C. and 24.8°C., and on the sole of one of the hind feet a temperature of 26.5°C. was noted. The average skin temperature of this rhinoceros was 26.2°C., a value slightly higher than the average found with the elephants, which was 25.5°C.

We were able to secure one measurement of the rectal temperature of this rhinoceros, namely, 37.4°C. (99.3°F.), which compares very well with Doctor Blair's records for the Indian rhinoceros of 100°, 99.7° and 99.7°F.

The skin temperature of a male hippopotamus. A specimen of the *Hippopotamus amphibius* (*Linn.*), a male, was also studied. This animal was 18 years old, had an estimated weight of 4600 pounds, and was 4 feet 10 inches tall, 11 feet 6 inches long, and 4 feet 3 inches broad. He was accustomed to spend much of his time in the water in a large tank adjoining his compartment, the temperature of which was approximately 15°C., but several hours prior to the taking of the temperature measurements the tank was drained and the animal was kept in a dry space along with his food. At the time of our measure-

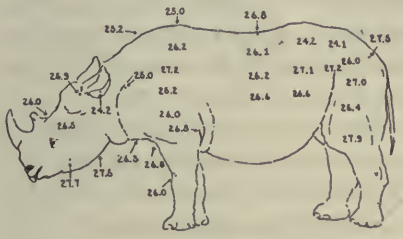

Fig. 4

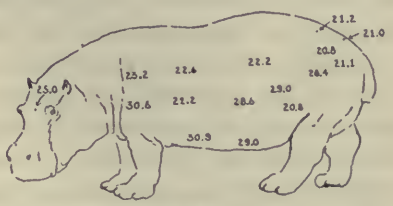

Fig. 5

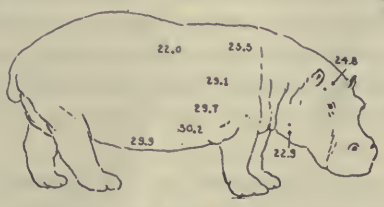

Fig. 6

Fig. 4. Surface temperatures of a female black African rhinoceros (left side). Temperature of environment, 19.5°C.

Fig. 5. Surface temperatures of a male hippopotamus (left side). Temperature of environment, 19.5°C.

Fig. 6. Surface temperatures of a male hippopotamus (right side). Temperature of environment, 19.5°C.

ments, therefore, the animal's body was reasonably dry, and while it felt slightly moist to the hand, no drops of water could be seen anywhere. The animal was extremely irritable and nervous, especially during the second series of observations; it was only with difficulty that we could secure the records here given, and we had to content ourselves with temperatures of the exposed skin, as it was impossible to measure the temperature in the groin, on the soles of the feet, or in any of the semi-enclosed cavities. The temperature topography of the hippopotamus is shown in figures 5 and 6 herewith, for the left and right sides, respectively.

From an inspection of the data in figures 5 and 6 it is clear that the skin temperatures are distinctly low as the back bone is approached, with a tendency for higher values down the side and under the belly. Even here the picture is not perfectly clear, since one extremely low value of 21.2°C. is noted near two high values of 30.6° and 30.9°C. The evidence is sufficient, however, to show that the lower half of the side of this animal has a relatively high temperature. It is not impossible that the lower temperatures on the upper part of the side are due to the vaporization of moisture from the skin and likewise to the possible downward drafts of cold air from the skylight and the ventilators in the chamber. These drafts of air were not sufficient, however, to affect the temperature of the wet and dry bulb thermometer, which remained essentially constant throughout the entire 3-day test.

From the relatively few measurements here recorded in figures 5 and 6 it is somewhat hazardous to estimate an average skin temperature for this animal. But if we follow the practice carried out with the elephants and rhinoceros and assume the legitimacy of averaging these results, we find the average for this animal is not far from 25.0°C. The hippopotamus, therefore, has an average skin temperature essentially that of the other three animals studied, although very much greater differences were observed between the temperatures of the back and belly than were found with either of the two elephants or the rhinoceros.

The irritability and viciousness of this particular animal finally resulted in the complete demolition of certain parts of our apparatus, which prevented further readings. No rectal temperatures could be secured with the animal, and the feces were not of sufficient volume and consistency to make it possible to secure the temperature of the feces immediately after their passage. It is probably true, however, that the rectal temperature of the hippopotamus is not far from that of the other large animals we studied, i.e., essentially that of man.

Suggestions for further study. All of the diagrams show that an attempt was made to measure the temperature at a spot corresponding approximately to the forehead of man, which always has a temperature much higher than other exposed parts of the body. With all of these animals relatively low rather than high forehead temperatures were recorded. This brings up the interesting question as to the influence upon the forehead temperature of the supply of blood to the brain, this factor undoubtedly being complicated by the extraordinarily thick skull and very thick skin of these pachyderms. Almost nothing

is known with regard to the blood supply and arterial distribution of blood in these large animals and the importance and significance of a thick skin as protection against heat loss, nor indeed is anything yet known with regard to their total metabolism and heat production per unit of weight or per unit of surface area. Measurements of this type involve very large and elaborate apparatus and partake of the nature of an engineering rather than a physiological problem. Aside from expense, however, no technical difficulties stand in the way of actual measurements of this type, although undoubtedly indirect calorimetric determinations of the carbon-dioxide production and oxygen consumption will have to be made rather than direct calorimetric measurements. But whatever the heat production, undoubtedly the extremely thick skin of these animals plays an important rôle in the protection against heat loss.

It is to be hoped that at some time a series of temperature measurements may be made with hairless animals of the types here studied, when the environmental temperature is measurably higher and lower than 19.5°C. The higher environmental temperatures could readily be obtained in certain seasons of the year, but the practicability of subjecting these tropical animals to a temperature much lower than 19.5°C. without danger is a matter of much uncertainty.

SUMMARY

Measurements were made of the skin temperatures of a group of large captive, hairless animals, namely, two elephants, a rhinoceros and a hippopotamus. For a number of years these animals had been subjected to the same routine of life in a well-ordered animal house at the New York Zoölogical Park and had become thoroughly acclimated to the conditions. For several weeks, if not months, prior to this investigation they had been living in an environmental temperature essentially constant, i.e., 19.5°C., and with no great changes in the degree of humidity.

With the two elephants the average temperature of the skin was 25.5°C. Local conditions of environmental temperature and drafts affected the skin temperature. Very pronounced temperature gradients were observed on various parts of the ears and extraordinary temperature differences were found at the tips of the right and left ears, both on the front and back of the ears and on different days. Of the three types of animals studied the elephants were by far the most restless and

much more active, for example, than the rhinoceros, who remained standing still or lying down practically the entire time, while the elephants were standing up and continually in motion. It is well known that with humans during severe muscular work there is an increase in skin temperature. On the basis of increased activity resulting in increased metabolism, one would, therefore, normally have expected to find a slightly higher skin temperature with the elephants than with the rhinoceros, but the actual records show exactly the opposite. With the rhinoceros the average skin temperature, 26.2°C., is somewhat higher than with the male elephant. In the semi-enclosed places, such as the groin and axilla and, especially with the rhinoceros, between the folds of skin, very much higher temperatures are observed, a value as high as 33.4°C. being found with the rhinoceros well up in the groin. The hippopotamus, by reason of its moist skin and amphibious nature, has a very widely varying skin temperature. Its skin is considerably colder on the back than on the belly. The temperament of this animal made it difficult to secure a sufficient number of measurements, but a rough average gives a value for the skin temperature of 25°C., which is not far from that found with the elephants.

All these animals, therefore, may be said in general to have essentially the same skin temperature, averaging about 25.5°C., or about 6°C. above the environmental temperature of 19.5°C. The rectal temperature is essentially that of man. The fact that all four animals show approximately the same average skin temperature is of special significance and leads to the interesting query as to what would be the skin temperature of a man who remained nude and in an environmental temperature of 19.5°C. for a considerable length of time.

STUDIES ON THE RESPONSES OF THE CIRCULATION TO LOW OXYGEN TENSION

V. Stages in the Loss of Function of the Rhythm Producing and the Conducting Tissue of the Human Heart During Anoxemia

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Received for publication April 9, 1921

That systemic asphyxiation is a factor in producing slowing of the heart rhythm and in decreasing conductivity has long been known,—Klug (1), Konow and Stenbeck (2) and numerous later investigators. Our present views as to the anatomical differentiations within the heart have resulted from a series of papers presenting the morphological and physiological facts of the conducting and nodal system,—Kent (3), His (4), Keith and Flack (5), DeWitt (6), Mall (7), Lhamon (8), Eyster and Meek (9) and many others. The facts of the functional control of the heart rhythm and sequence from dominant centers as understood at the present time have been developed especially by Erlanger (10), Keith and Flack (5), Adam (11), Flack (12), Lewis (13), Ganter and Zahn (14), Meek and Eyster (15) and Lewis (16). The influence of temperature, of asphyxia, of drugs, and especially of the extrinsic nerves on the sino-auricular and auriculo-ventricular rhythms and on conduction have been discussed in several of the preceding references and also by McWilliam (17), Lewis and Mathison (18), Mathison (19), Meek and Eyster (20), Eyster and Meek (21), Schlomovitz, Eyster and Meek (22), and by Lewis, White and Meakins (23). The reference list is not exhaustive but the literature is fully reviewed in several of the references given. We have also briefly reviewed the literature on asphyxia in article III of this series (24).

The electrocardiographic method has been applied to the study of the changes in the mammalian heart in numerous studies from the laboratories of the University of Wisconsin by Eyster and Meek,

who used also the method of asphyxia to follow the point of origin of the pace-maker of the dying mammalian heart. In Lewis' laboratory in London, too, numerous electrocardiographic studies have tended to clarify our knowledge of the physiology both of the factors of intrinsic and extrinsic cardiac regulative control. Lewis and Mathison (18) showed that conduction and rhythm production are decreased even to the point of suspension when an animal with open chest is allowed to asphyxiate by stopping artificial respiration, and they have published electrocardiograms showing these points. Ganter and Zahn (14) and Meek and Eyster (21) found that if the sino-auricular node was cooled locally the pace-maker was driven to a lower point in the heart, i.e., the auriculo-ventricular node. This the latter proved by two leads taken directly from the exposed heart. Lewis, White and Meakins (23) showed that the displacement was by gradual steps in some animals, the cat, and by sudden shifts in others, the dog, as shown by shortening of the P-R interval. They observed that when the rhythm arises in the A-V node the conduction may be retrograde, as indicated by the R-P interval, a new point in mammalian heart physiology. Further asphyxiation suppressed the retrograde conduction and all evidence of auricular contractions disappeared. We call attention to the experiment of Lewis, Meakins and White on the cat, figure 6, plate 2, with the following legend: "After establishing A-V rhythm by applying cold continuously, a cat was asphyxiated; by the 145th second of asphyxia the heart passed through several stages of partial reversed block and finally the auricular contractions had vanished. The curve commences at this state and shows the recovery of the S-A node soon after the withdrawal of cold from it. The ventricular rate is practically unaltered and complete heart-block is evidenced by the dissociation of auricular and ventricular rhythms." This figure is quoted as direct experimental proof of asphyxial suppression of auricular contractions in the mammalian heart.

In our previous papers we have shown that in men during extreme oxygen-want very marked changes may occur in the mechanism of the human heart. These critical changes for the most part did not appear until the stage of oxygen-want in which the oxygen is insufficient to maintain the nervous system in conscious activity. In other words, the more profound changes during low oxygen are imminent at the approach of unconsciousness, and of skeletal muscular collapse, though cardiac slowing and disturbance of the normal mechanism follow these events in sequence. We have published examples in which two of the chief

irregularities of the human heart are loss of auricular contraction as evidenced by disappearance of the P wave, and loss of internodal conduction as indicated by dissociation. In the present paper we give in detail the reactions of an extreme case of this type, a case somewhat different but crucial as regards the nature and sequence of the local cardiac changes which we have followed without interruption.

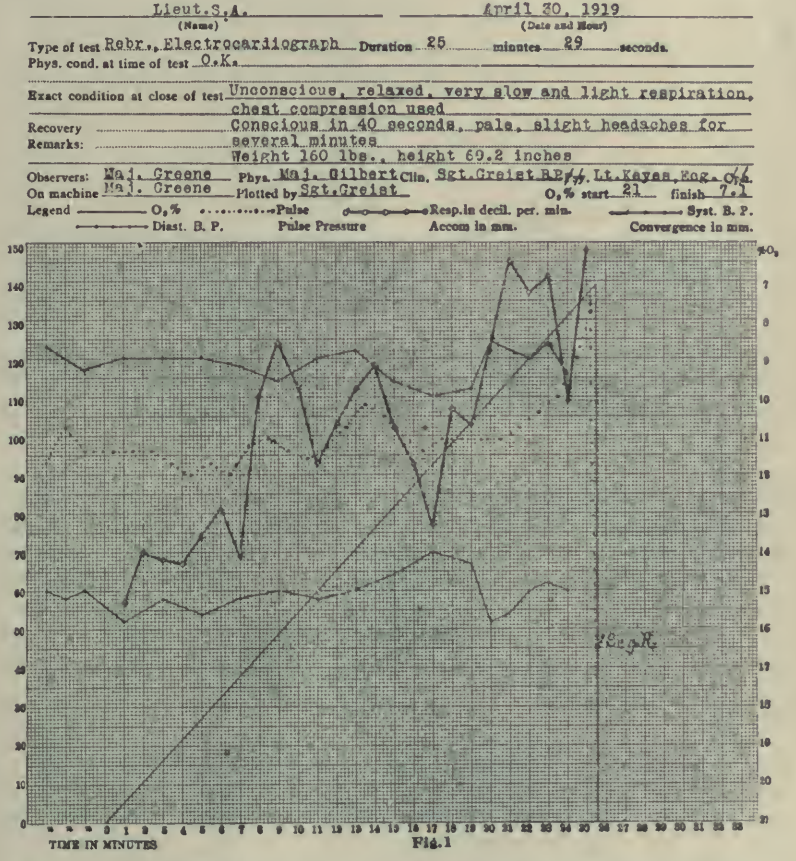

Fig. 1

Fig. 1. The clinical chart of Lieut. S. A. shows the heart rate, dotted line; systolic blood pressure, the top light line; diastolic blood pressure, the bottom line; respiratory minute-volumes, heavy line. The heart rates are taken by 20-second counts at the wrist during the first 20 minutes, and from the electrocardiogram from the 20th minute to the end of the record. The blood pressures are measured by the Rogers sphygmomanometer. The deciliters of air breathed per minute were read off the Larsen recorder. The blood pressure, heart rate and decileters of air are all shown by the legend to the left; oxygen percentages are indicated to the right.

Fig. 2. The heart rate per minute of Lieut. S. A. during the post-crisis stage of oxygen-want, calculated beat by beat beginning with the 26th minute. *Unc*, became unconscious at about this point; *off*, removed from the test.

TABLE 1

Showing the variation in the pulse and in the electrocardiogram at intervals through the rebreather test. Time in seconds. Amplitude of the deflection in millimeters, equivalent to 10^{-4} millivolts. Lieut. S. A. April 30, 1919. Final oxygen 7.1 per cent. Time of run 25 minutes, 29 seconds. Equivalent altitude 28,000 feet

TRACE AND PULSE NUMBER	TIME IN MINUTES	OXYGEN IN PER CENT	DURATION IN SECONDS			AMPLITUDE IN MILLIMETERS				
			R-R	P-R	R-T	P	Q	R	S	T
1-10	Normal	21.0	0.656	0 136	0.320	0.8	None	14.0	1.0	2.4
2-4	5	18.2	0.648	0.140	0.328	1.0	None	13.5	1.2	2.3
3-2	10	15.5	0.624	0.140	0.292	0.9	None	15.0	1.6	1.8
4-1	15	12.7	0.640	0.152	0.312	0.9	None	13.0	1.4	1.8
5-20	20	10.0	0.584	0.144	0.280	1.0	None	12.0	1.5	1.6
6-122	22	9.0	0.700	0.152	0.296	1.0	None	11.5	1.0	1.8
7-237	24	7.9	0.568	0.136	0.280	0.9	None	12.0	1.4	1.0
8-275	25	7.3*	0.492	0.120	0.260	1.2	None	9.5	1.4	1.0

* Circulatory break at the end, see table 2. No recovery tracing secured.

TABLE 2

Variation in heart rate and in the electrocardiograms through the entire post-crisis stage of Lieut. S. A. The rates are computed on the basis of the length of the R–R intervals beginning at the crest of the maximum heart rate. The break occurred at 25 minutes, 10 seconds. The measurements of the P–R and R–T intervals are difficult and the factor of error is large. Lieut. S. A. April 30, 1919. Time of run 25 minutes, 29 seconds. Final oxygen 7.1 per cent—28,000 feet elevation.

TIME IN SECONDS BEGINNING AT 25 MINUTES	EQUIVALENT RATE FOR EACH CONSECUTIVE BEAT		ELECTROCARDIOGRAMS			REMARKS
	Pulse number	Rate	P-R interval	R-T interval	R amplitude	
10.00	1	132	0.13	0.26	11.5	
10.48	2	132	0.13	0.26	12.6	
10.90	3	129	0.13	0.26	12.4	
11.38	4	131	0.13	0.25	12.4	
11.84	5	129	0.13	0.27	12.6	
12.32	6	125	0.13	0.28	12.6	
12.80	7	121	0.13	0.29	13.0	
13.30	8	118	0.13	0.30	13.0	
13.32	9	116	0.14	0.31	12.4	
14.36	10	110	0.13	0.29	13.8	
14.96	11	105	0.12	0.30	14.0	
15.54	12	102	0.13	0.32	14.5	Muscular tremors severe during four beats
16.12	13	97	0.14	—	14.0	
16.80	14	91	0.13	0.33	13.4	
17.54	15	81	0.12	0.33	13.4	
18.48	16	63	0.112	0.33	13.0	P inverted
19.36	17	66	0.098	0.31	13.6	P inverted
20.28	18	66	0.094	0.30	13.0	P inverted
21.18	19	65	0.10	0.30	14.6	P inverted
22.30	20	54	0.10	0.34	14.0	P inverted
23.40	21	54	0.10	0.30	16.0	P inverted
24.68	22	46	0.10	0.31	16.0	P inverted
26.04	23	45	0.17	0.35	22.6	R–P or reversed conduction the P still inverted
27.36	24	45	0.21	0.33	22.3	
28.72	25	44	0.23	0.34	21.5	"Off" here
30.04	26	45	None	?	18.4	No P waves during the 10 recorded beats of this stage
31.26	27	45	None	?	16.5	
32.68	28	46	None	?	19.0	
34.00	29	48	None	?	18.0	
35.28	30	47	None	?	19.4	
36.58	31	47	None	?	18.0	
37.82	32	48	None	?	16.5	

Lieut. S. A. was carried to the stage of unconsciousness in an altitude test by the rebreather method, the procedure being the same as used in our previous research. Electrocardiograms were obtained at 5 minute intervals to 20 minutes, then a continuous electrocardiogram was recorded for the last 6 minutes and to the close of the test. The graph representing clinical progress of the entire test is shown in figure 1. The general electrocardiographic changes throughout the test are given in table 1. The heart rates calculated on the basis of the time of the successive cycles from a moment before the maxium heart rate through the terminal fall in rate to the end of the test are shown in figure 2. The electrocardiographic data for the critical post-crisis period is presented in table 2. The electrocardiograms representing the successive stages of the test are reproduced in plate 1, figures 1 to 8.

The general changes during the development of low oxygen are not essentially different from the types previously described. Lieut. S. A. follows the rule during the pre-crisis stage, both in the clinical cardio-vascular responses and in the changes shown by the electrocardiograms. The heart accelerated to 136 at the crisis, but with considerable variation in time of cycles between the 12th and 17th minutes. The systolic pressure fell during the 13th to 18th minutes, which, taken with the rise in diastolic pressure, the change in heart rate and the fall in respiratory volume, indicates a failure to adequately respond to the strain at this period. He rallied and continued to compensate until the 23rd minute and became unconscious in the 25th minute. These irregularities of compensation characterize nervousness during the test rather than weakness under the test. However, the test was made to the extreme limits and thus revealed vital and illuminating post-crisis changes in the heart.

Unconsciousness supervened at the point marked *Unc* in the electrocardiographic record, figure 8. He was continued in the test until his muscles began to relax, i.e., 10 seconds after the evidence of unconsciousness, and was then rapidly removed from the rebreather. Skeletal muscle relaxation became complete during removal of the mouth-

Plate I. The electrocardiogram of Lieut. S. A.; figure 1, normal; figure 2, after 5 minutes; figure 3, 10 minutes; figure 4, 15 minutes; figure 5, 20 minutes; figure 6, 22 minutes; figure 7, 24 minutes; figure 8, begins at 26 minutes and 10 seconds; figure 9, continuation of figure 8. The four shadows on figure 8 and the word *off* mark the point when Lieut. A. was taken off the test. The changes in heart rate in figures 8 and 9 are shown graphically in text figure 2. Time in fifth seconds. The R deflections of the original record have been strengthened by the engraver in reproducing the plate.

Fig. 1
Normal, pure air

Fig. 2
18.2 oxygen

Fig. 3
15.5 oxygen

Fig. 4
12.7 oxygen

Fig. 5
10.0 oxygen

Fig. 6
8.9 oxygen

Fig. 7
7.8 oxygen

Fig. 8. 7.2 oxygen

Fig. 9. Continuation of figure 8

piece, and relaxation continued to the end of the cardiographic record. His respirations at this time were very shallow and faint. Artificial respiration by compression of the thorax was produced at this point. Unfortunately at this critical time the electrocardiographic apparatus became disconnected so that the transitional stages toward cardiac recovery were not recorded. He remained unconscious about 40 seconds, then quickly and suddenly regained consciousness and muscular control. He was a little pale, was not nauseated, suffered some slight headache for several minutes, but otherwise showed no deleterious after-effects from the experience.

Both the clinical determinations and the heart rates obtained from the electrocardiograms show early progressive acceleration of the heart. This reaction is usually given by a normal compensator during the early and pre-crisis stages of the altitude test. The heart rate augmented from the rather high initial rate of 90 to a maximum of 136 per minute at the crisis at the beginning of the 25th minute. At this time the rate began to decrease, at first slowly through 6 or 7 beats, then very rapidly, until the low rate of 44 per minute was reached when the test was terminated. The beats remained at the slow rate for the remaining 10 seconds recorded.

The electrocardiogram taken continuously from the 20th minute, therefore including the time from the moment of maximum heart rate through the entire terminal or post-crisis period, shows in continuous panorama the facts on which we base the interpretation of the extreme effects of low oxygen on the behavior of the normal human heart. We emphasize above all the evidence that oxygen deficiency does not become vital to the heart itself until late, certainly not before the onset of unconsciousness. But when the crisis is reached the post-crisis changes occur rapidly, indeed in a few seconds.

The graph, figure 2, and the electrocardiogram, figures 8 and 9 of plate I, show that during 20 consecutive beats the rate dropped from 136 to 44 per minute. The rate was 100 at the beginning of a group of 4 contractions complicated in the electrocardiographic record by skeletal muscular tremors. Unconsciousness occurred during this period, according to the clinical evidence on which we base judgment. Lieut. S. A. did not immediately lose reflex control of his muscles, and still held the mouthpiece safely for a few seconds longer. The rate was 81 at the inversion of the P and the onset of unconsciousness and 66 at its consummation.

An inverted P wave appears during the last heart beat of the group in which skeletal muscular contractions occurred. This beat is coinci-

dent with a decided slowing in the rate, from 102 just preceding the unconscious stage to 64 during this beat. There is also a sudden reduction in the P-R interval showing that the time of conduction from the new source of the beat to the ventricular tissue is reduced, from 0.127 to 0.098 second (see table 2). Inversion of the P wave and shortening of the P-R interval are interpreted as signifying origin of the rhythm lower down in the system, i.e., the S-A node, or at least as low as the coronary sinus portion of the A-V node, as indicated in certain displacements observed by Ganton and Zahn, and by Meek and Eyster. The inverted P exists through seven successive contractions though the third contraction does not show the phenomenon clearly. In these seven beats the P wave precedes the R by a very uniform but shortened time interval.

On the third beat before the close of the test the P wave suddenly shifts to a post R position with a relatively long R-P interval. The last beat of the three in which this condition exists occurs at the moment of taking "off." Each successive R-P interval is longer, 0.17, 0.21 and 0.28 second, signifying the progressive and rapid loss of reverse conduction. This group of contractions indicates the shift of the rhythmic center to a still lower or third point of origin, presumably low in the A-V node. The sequence is reversed and conduction is back to the auricle. At this moment the rate is at its slowest, i.e., 44 to 45 per minute, where it remains with little variation during the last 10 beats recorded.

Augmentation in the amplitude of the R wave takes place at the time when the P shifts to the post R position. We offer no obvious explanation for this fact other than that the ventricle beats first in sequence, i.e., is the primary activity. During the last 10 beats the amplitude of the R varies little or none from beat to beat. The irregularity of the record from extrinsic currents confuses the R-S-T amplitudes to a degree.

The steps in the transition of the P outlined above indicate the suppression of the successive pace-making foci. The normal S-A node first succumbs during asphyxiation. The first ectopic rhythmic focus next ceases to function. There is finally left a rhythmic center well down in the conducting system adequate to maintain rhythm. It is true the rhythm is at a greatly reduced rate although the reduction is not so great as that often observed in pathological heart block.

At the moment of closing the experiment the P wave disappeared entirely. It did not reappear during the ten ventricular complexes of the remainder of the record. This we interpret as suppression of func-

tion of the conducting tissue sufficient to block conduction from the lower rhythmic center back to the auricle, demonstrating for man the additional new point that anoxemia blocks retrograde conduction from the ventricle toward the auricle during A-V rhythm. This was first shown to be true during systemic asphyxiation by the method of stopping respiration in lower animals by Lewis, White and Meakins who of course were dealing with excess of carbon dioxide as well as lack of oxygen. All the remaining contractions are normal ventricular complexes of the type in which the beat arises in the conducting system rather than in the muscle. No ectopic beats occurred in this case.

There is only the merest suggestion of an increase in rhythm during the ten beats that occur without auricular beats, in fact the rate is slow and remarkably regular from the moment the first R-P interval occurs.

This record gives conclusive evidence from the human subject that the pace-making function in the heart is depressed and lost in the descending direction during anoxemia induced by the rebreather method. The evidence from mammalian experimental sources is confirmed for the human. The most sensitive parts of the conducting system are the S-A node and the internodal region. These are both rendered inactive by oxygen-want long before rhythm and conduction are lost in the A-V node and the peripheral parts of the system. We believe these changes are immediately due to vago-spasm. But whether one accepts the explanation of vagal stimulation or of direct asphyxial effects, it is obvious that when for any reason, either physiological or pathological, the S-A pace-making center ceases to function and the auricular or internodal paths cease to conduct, thus suppressing auricular contractions, then the basic rhythmic property of the A-V node still persists to control the contractions of the ventricle for a time until the crisis passes. This control proceeds from the A-V node over the ventricle, and the rate though slow is adequate for a circulation of considerable efficiency. These observations agree with the deductions made in our previous paper. They confirm for man the facts pointed out by Meek and Eyster (20) for the dog, i.e., that the heart is sensitive to extrinsic control in the descending direction and "that the specialized tissues of the heart exhibit from above downward progressively diminishing degrees of automaticity."

The case of Lieut. S.A. differs from the others reported by us in that the shift of the point of origin of the rhythm and the loss of internodal conduction is by relatively sudden steps, rather than by progressive displacement of the pace-maker and of conduction.

The facts observed in our case are clear-cut and definite and we recognize that the practical significance in aviation and in clinical medicine is definite and clear. The slowing of the rhythm, inversion of the sequence to ventricle—auricle beats, and finally the decrease and disappearance of auriculo-ventricular conduction are all changes perfectly characteristic of over-activity of the vagus center. This human case might be explained on the basis of Mathison's observations of mammalian asphyxial vago-spasm. Wilson has shown that the S-A node is particularly sensitive to vagal control in man both in the normal (26), and in the stimulative stage of the action of atropine (27). Vagus action not only changes auricular rate but lowers auricular intensity, as shown by decrease in amplitude of the P. Change in amplitude of the P was not observed in Lieut. S.A. The P was inverted—not the usual type of vagus effect. The ventricular rate is most constant. In view of the evidence of direct vagal inhibitory influence over the A-V nodal rhythm, the failure of further ventricular slowing during the extreme anoxemia needs explanation on the vago-spasm hypothesis. However, we believe that the vagus stimulation hypothesis offers the most convincing explanation and is confirmed by this human case.

In the meantime we have in progress comparative experiments on mammals based on the rebreather-electrocardiographic methods which we hope will throw further light on the nature of the final post-crisis reactions of progressive oxygen-want as observed in man.

In closing this paper we again emphasize the numerous parallels we have observed in the physiology of the normal nodal system of the human heart in comparison with the facts of cardiography established on experimental animals. We also emphasize the correspondence as between simple anoxemia and the symptom complex of asphyxial cessation of breathing when excess of carbon dioxide is added to anoxemia.

SUMMARY

An additional and extreme case of oxygen deficiency on the normal human subject showing changes in the heart during a rebreather test is presented with continuous electrocardiograms through the crisis and post-crisis periods. The data show:

1. That reflex muscular control in the human may persist 6 to 8 seconds after loss of consciousness from anoxemia.

2. That sino-auricular rhythm is lost by steps first to a lower point in the sino-auricular system, second to a point nearer the base of the ventricle, presumably the auriculo-ventricular node.

3. That internodal conduction is finally lost a brief stage of reversed conduction terminating in lengthening R-P intervals precedes total loss of conduction.

4. That the ventricular rhythm is very persistent and unexpectedly regular during the late post-crisis stage. In this case the equivalent rates are from 63 to 44 increasing to 48 per minute in 10 seconds after removal of the mouth piece.

5. That in man lack of oxygen induces a series of changes in cardiac rhythm, in conduction and in suppression of auricular contractions quite parallel to similar phenomena established in experimental animals under general asphyxiation.

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OSCILLATIONS IN DIAPHRAGM MUSCLE

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Received for publication April 11, 1921

That oscillations appear in diaphragm muscle under various conditions has long been known. Mosso observed them in dogs and rabbits in normal sleep and under anesthesia (1). We have observed these oscillations in urethanized dogs, cats and rabbits and in decerebrate cats and rabbits (2). The reason for their appearance does not seem to have attracted much attention. In searching through the literature we have been unable to find data to locate their exact cause. There seems, however, to be a general consensus of opinion that they are due to central origin (3). In an attempt to find out whether these oscillations may not be due in part to peripheral origin, the following experiments were undertaken.

METHODS

The animals used in this investigation were pithed cats and dogs which were previously anesthetised with ether or with urethane (2 grams per kilo of body weight) by stomach. At times ether was also administered to the urethanized animals to hasten the anesthesia before operating. An animal was fastened back downward to an animal holder, a cannula inserted into a femoral artery, and a mercury manometer attached for recording the blood pressure which was used as an indicator for the general condition of the preparation and for comparison with contractions of the diaphragm muscle.

The abdominal cavity was opened by a median incision and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall as previously used by Nice and his co-workers (4). From the S-shaped hook a thread was passed over a pulley to a light writing lever which recorded the contractions of the diaphragm on a revolving drum. In some of the experiments a plethysmograph record was also made of the chest wall but no change in the record took place so it was omitted later.

In the earlier experiments the urethanized animals were permitted to write simultaneous records of their blood pressure and movements of the diaphragm until oscillatory waves appeared in the latter; but soon this was found unnecessary so the stimuli were applied at once. A tracheal cannula for artificial respiration was inserted, both phrenic nerves severed in the neck, and a Harvard shielded electrode placed on the peripheral end of a transected phrenic on the same side on which the S-shaped hook had previously been attached to the diaphragm. Both carotid arteries were tied off and the cord transected through the foramen magnum and the brain pithed. Artificial respiration was applied for from 2 to 5 minutes and then the peripheral end of a cut phrenic nerve was stimulated with a series of break-induction shocks. Artificial respiration was again applied and a new series of break induction shocks sent into the nerve and so on. The temperature of the animal was maintained by an electric heater.

The stimulating current for the phrenic nerve. The stimuli used for the peripheral end of the phrenic nerve were a series of break induction shocks obtained from a vulcanite key which is a modification of the original one described by Martin (5). The key was operated by a motor running at a uniform rate. The number of stimuli used varied from 120 to 188 per minute, however, 148 was the usual number employed, yet the other figures seemed equally satisfactory. The strength of the stimulus was the same throughout a single experiment.

RESULTS

Types of oscillations. In taking up the results of this work it should be stated that oscillations appeared most prominently in vigorous, well-nourished animals while they failed to appear only in a few asthenic ones. Another fact that should be noted is that these oscillations may appear and disappear or take an entirely different form under the same strength of stimulus, and the amplitude of the curve may vary greatly.

In figure 1 the peripheral end of the left phrenic of a pithed dog was stimulated 148 times per minute. It is seen that the oscillations show great variation. At *B* the rate of speed of the drum was increased to get the individual variations in the single contractions of the muscle. Oscillations similar to those in the first part of the curve *A* reappear. Figure 2, which was taken on the same animal about 30 minutes later than figure 1, shows more marked oscillations.

Figure 3 was obtained from the right side of the diaphragm muscle in a decerebrate cat. In *A* the typical oscillations that appear are seen while *B* is a continuation of the stimulations in *A* after an intermission of 2 minutes during which time artificial respiration had been administered. Although the strength of stimulus and the rate of stimulation were the same as in *A*, the muscle curve in *B* shows no oscillations.

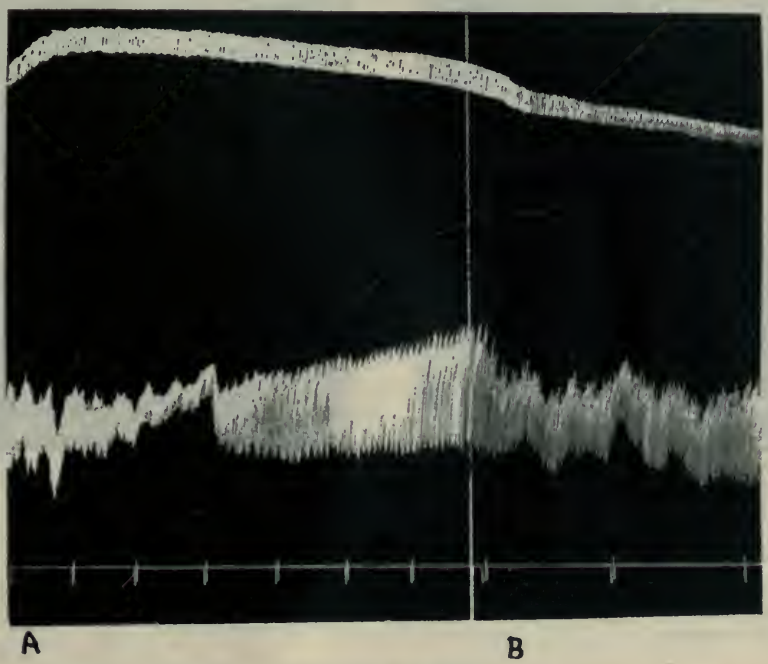

Fig. 1. Dog A. In this and all following records the upper curve indicates the blood pressure; the middle curve the contractions of the diaphragm; and the lower line the time in half-minutes. The left phrenic nerve was stimulated with break induction shocks at the rate of 148 per minute. In *B*, which is a continuation of *A*, the rate of speed of the drum was increased.

This is a common occurrence in these experiments. We have also observed that oscillations may be obtained on one half of the diaphragm and refuse to appear on the opposite side.

Relations of the oscillations to blood pressure. The blood pressure in pithed animals is known to be low. In figure 1 and figure 2 there are no changes in blood pressure from the level that can account for the oscil-

lations in the diaphragm muscle. In figure 3, *A*, there are some variations in blood pressure but not of sufficient size, it would seem, to account for the changes in the curve of the diaphragm muscle.

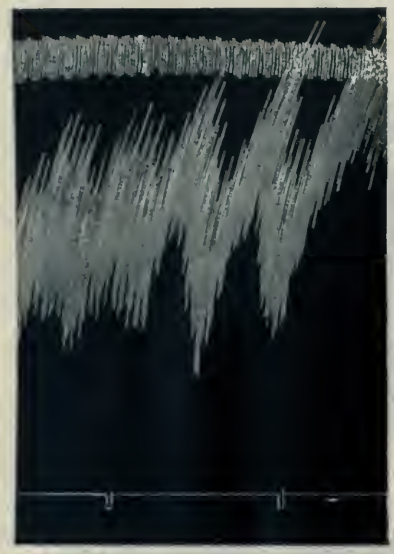

Fig. 2

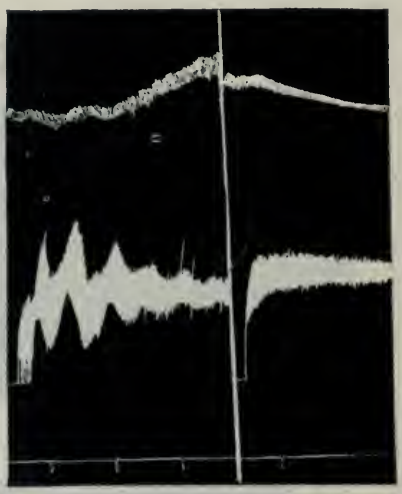

Fig. 3

Fig. 2. Dog A. This record was obtained on the same animal as figure 1 about 30 minutes later.

Fig. 3. Cat. Oscillations in the right half of the diaphragm Phrenic nerve stimulated 176 times per minute. *B* is a continuation of *A* after an intermission of 2 minutes.

DISCUSSION OF RESULTS

Diaphragm muscle is unlike some other striated muscles in that it is more resistant to fatigue, more resistant to death after excision from the body, and is less injured in inanition (5).

Relation of blood pressure. In pithed animals the blood pressure is low yet these oscillations appear on stimulation of the peripheral end of a cut phrenic nerve.

Cannon and Gruber (6) have shown that oscillatory waves appear in the tibialis anticus muscle of the cat in pithed animals and also in excised perfused muscle under rhythmic Faradic stimulation so that blood pressure seems not to be a factor in these cases.

Source of oscillations. Oscillations in diaphragm muscle are generally considered to be of central origin (7). Our results show that they appear when the nerves from the central nervous system are severed and the peripheral end of a phrenic is stimulated with break induction shocks.

Lee and his co-workers found "rhythmicity" appearing in isolated diaphragm muscle of the cat when it was stimulated directly at a uniform rate (8). The question arises as to whether this "rhythmicity" may not be a characteristic of the muscle itself that has given rise to the oscillations in our experiments with the muscle in situ.

Since the upper cord was pithed and both phrenic nerves transected, these oscillatory waves must be of peripheral origin and would seem to be due to changes in the irritability of the muscle itself.

SUMMARY

1. Oscillations in diaphragm muscle similar to those occurring in animals in normal sleep and under anesthesia and in decerebrate cats and rabbits may appear when the cord is pithed in the cervical region, the brain destroyed, both phrenic nerves transected and the peripheral end of one stimulated with break induction shocks.

2. These oscillations take different forms and may appear, disappear and re-appear under the same strength of stimulus.

3. There seems to be no relation between changes in blood pressure and the character of the oscillations in diaphragm muscle in pithed animals.

4. The evidence indicates that the oscillations in our experiments are due to changes in the irritability of the diaphragm muscle itself.

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BALTIMORE, MD.
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PHYSIOLOGICAL ACTION CURRENTS IN THE PHRENIC NERVE. AN APPLICATION OF THE THERMIONIC VACUUM TUBE TO NERVE PHYSIOLOGY[1]

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Received for publication April 9, 1921

Many experimenters who have had experience with the recording of the action currents of nerve have felt the need of more sensitive apparatus than has been available. The development of the three electrode vacuum tube has suggested independently to a number of physiologists that this instrument might be of great value in the elucidation of problems in electro-physiology. It was first suggested to one of the present authors in the spring of 1919 in a conversation with Mr. A. S. Langsdorf. Since we have been working on this problem three papers have appeared on the application of the vacuum tube to physiology. Höber (1) repeated the older observations on muscle made by Bernstein and Schoenlein, and by Wedenski with a telephone, bringing to his aid a vacuum tube cascade. Little description of his instrument is given. From the diagram it appears to be a three tube transformer coupled cascade connected to input and output also by transformers. The three filaments were operated from one battery as were also the three plate circuits. No graphic methods were used; the author states, however, that the apparatus was noisy.

[1] Both amplifiers used were built by Doctor Newcomer at the Pennsylvania Hospital. The experiments were jointly carried out at the Washington University.

Forbes and Thacher (2) have described a very serviceable arrangement by which the action current of nerve may be amplified and recorded with the string galvanometer. For this they used one tube with the output connected to the galvanometer through a condenser. They also made a very detailed study of their circuit in the search for the conditions giving optimum amplification. Recently Daly and Shellshear (3) have described an apparatus in which the tubes were each placed in one arm of a Wheatstone bridge. They found three valves in cascade were necessary to give convenient amplification. Their apparatus has the advantage of aperiodicity, but is not, however, in its present form, of value for nerve studies on account of the large amount of adventitious vibration appearing in their records.

The apparatus used in our first experiments (June, 1920) consisted in an alternative one, two or three stage amplifier, resistance coupled when used in cascade. The input could be connected either directly or

Fig. 1. Action currents from the phrenic nerve showing an admixture of "noise." The record was made with two tubes and both the input and output transformers. Time, $\frac{1}{5}$ second. Magnification in all records 500 diameters.

through a transformer; the output could be connected to the galvanometer either through a transformer or a condenser. One 6 volt storage cell served for all three filaments and one 150 volt dry cell battery operated all three plate circuits. As the result of the loss of strings in attempts to use the condenser output we confined our experiments at this time to the transformer. In the light of our subsequent experience these accidents seem in all probability to have been due to a leak between the string mounting and the ground.

As we were interested primarily in the recording of physiological action currents, the phrenic nerve was used as a test object. Good amplification was obtained with one or two tubes. Three tube amplification seemed to be more than was practicable. Figure 1 shows the action current obtained from the phrenic nerve during the latter part of inspiration and the beginning of expiration. Two tubes and the transformer input and output connections were used. It will be seen

from the record that the string was not quiet during the respiratory pause but shows a double rhythm, one of 63, the other of 380 per second. This rhythm is continuous and may be traced into the records of the action current.

As the result of these experiments it was apparent that the problem of major importance was to so change the design that the galvanometer string would be quiet when there were no input connections. The apparatus was accordingly rebuilt as will be described, and proved to be more satisfactory.

The nature of the central innervation of muscle was selected as a good type of investigation that could be improved by directing the attention to the action currents of the nerve as well as to those of the muscle. Most of the numerous investigations of this subject have been made on the action currents of muscle because means have been lacking for the ready study of nerve activated from the central nervous system.

Piper (4) led off the action currents from the forearm to the string galvanometer and obtained a rhythmic oscillation of the string at the rate of about 50 per second. He interpreted this as the rate of central innervation. This interpretation met with considerable opposition as it was known from previous work, and has been confirmed by more recent work, that the action currents of muscle do not necessarily follow the stimulus when applied either directly or indirectly through the nerve (5), (6), (7). Wedenski, who studied muscle by means of a telephone, found that the sound followed the rate of stimulation up to a limit and then the muscle responded with a lower tone. When the rate of stimulation was very fast the "bruit" in the telephone was very similar to the sound produced by voluntary innervation; he therefore ventured the opinion that the innervation rate of muscle may be very much higher than that obtained from the muscle.

Wedenski's view was supported by Buchanan and by Garten. It was found that a rhythmic response was obtained from muscle indistinguishable from that occurring in voluntary innervation, not only from stimuli of high frequency but from the tetanus producing stimulus which occurs from opening or closing a strong constant current applied to nerve or muscle. Von Kries (8) had previously shown that a prolonged stimulus from a rheotome gave both a longer contraction and action current than results from an induction shock and concluded that the natural stimulus to muscle may vary in duration as well as in strength and rate. These findings were confirmed by Hoffmann (9)

who, however, found as the result of more delicate methods that the muscle action current consisted, depending on the duration of the stimulus, of from one to five waves and that the contraction was therefore a short tetanus. In the light of experiments by Dittler and Oinuma (10) and Beritoff (11), the appearance of a rhythm in the action current, depending upon the nature and the condition of the tissue and partially independent of the rate of stimulation, when the tissue is stimulated by a rapidly oscillating or strong constant current, may be attributable to the refractory period of the tissue. In a later work Garten (12) found that the oscillatory action current of muscle produced by a closing tetanus is also present in the nerve. He then expressed the opinion that the nerve rhythm is of the same order of magnitude as the muscle rhythm and thereby allows the possibility that if the nerve rhythm is determined by the central nervous system, the muscle rhythm is centrally controlled.

The sum of the experiments on the action currents of muscle clearly shows that when a given rhythm is obtained by leading from muscle in voluntary activity to a galvanometer, no conclusion is possible as to whether or not this rhythm is also the rhythm in its nerve. It is also equally true that the intrinsic period (Eigenperiode) of the muscle is not revealed.

The question can only be settled by an examination of both the muscle and its nerve, preferably simultaneously. This was done by Dittler (13) in the case of the diaphragm. Action currents were taken from the diaphragm of the rabbit and cat and compared with those obtained from the phrenic nerve. In the latter case he was sufficiently successful on connecting the cut end and side of the nerve to the string galvanometer to obtain oscillations whose rate could be determined for a part of the respiratory cycle. The nerve rate was very close to that of the muscle. In a later investigation, Dittler and Garten (14) recorded with two large string galvanometers the action currents of the dog's phrenic simultaneously with the action currents of the corresponding side of the diaphragm. Under these conditions where the action currents were necessarily smaller than those obtained by a lead from the cut end and side of the nerve, they obtained small pointlike deviations in the string picture during inspiration, recognizable with a lens. In the more successful experiments they believed they were able to identify corresponding waves in the records of the phrenic and diaphragm but state that it would be desirable for a more exact judgment to obtain larger and therefore more convincing curves of the diphasic action current.

METHODS

The experiments were done on dogs under a morphine and ether anesthesia. A wide exposure of the phrenic nerve usually on the right side was made and the nerve dissected free from the vena cava, surrounding connective tissue and fat, then severed close to the diaphragm. This gives a long stretch of nerve which belongs entirely to one muscle. The nerve was fastened through three non-polarizable electrodes containing wool yarn that could be tied about the nerve. The third electrode was not filled with zinc sulphate but served merely as a support to prevent movements from being translated to the other two. All the electrodes were supported on a stand, free from the operating table to prevent vibration during respiration. The distal electrode was connected with the cut end of the nerve and the ground. The proximal was connected to the side of the nerve and to the grid of the first tube. By means of a double throw switch the preparation could be connected to the galvanometer either directly or through the amplifier. The artificial ventilation necessary on account of the open thorax was maintained to the point of apnea. The records were obtained during periods of temporary suspension of the artificial respiration.

For a discussion of amplifier design the reader is referred to a chapter on that subject by Van der Bijl (15). Figure 2 is a diagram of the amplifier used in these experiments. The essential parts were all manufactured by the Western Electric Company. The original design used in June, 1920, was adapted at their suggestion from that of one of their standard sets and Mr. R. E. Bitner of their Research Branch has since given us valuable suggestions, the most important of which was to reduce the adventitious vibrations by using separate batteries for each tube. The input is connected to the grid circuit either directly or through a transformer. In the first instance the grid circuit is shunted with a resistance of 2 million ohms, in the latter with one of 100,000 ohms. The transformer has four taps permitting one to vary with the switch, S_1, the input impedence of the circuit to an optimum. Each tube is operated by a separate plate battery of 150 to 160 volts consisting of no. 734 Eveready dry cells. The filaments are operated separately by three storage batteries of 6 volts each. Two resistances of 500,000 ohms each are inserted between the second and third grids and the ground. These serve to maintain the grids negative to the filaments because of the potential drop in the filament resistances of the filament circuits. In the plate circuit of each tube is inserted a

resistance of 200,000 ohms separately shielded. The second and third grids are protected from the potentials of the preceding plates by the insertion of condensers each having a capacity of 2 microfarads. Their resistance is 4 billion ohms. The tube and the plate battery act as a generator of alternating potential because of variations in the tube resistance. This potential is applied across the circuit consisting of the coupling condenser and the 500,000 ohm resistance and thus varies the potential of the next grid. The alternating current output of

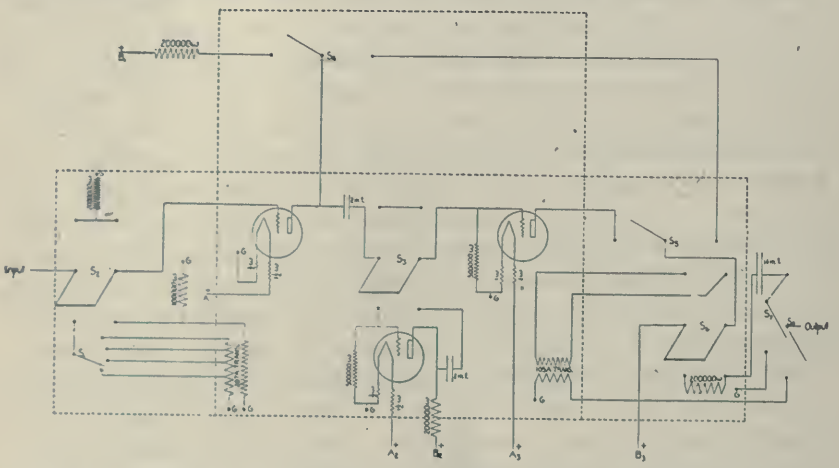

Fig. 2. The dotted lines represent the outlines of the metallic shield, the sides and back being thrown open to show the wiring. The points G indicate the grounding of a wire to the shield. The three A batteries of 6 volts each and the three B batteries of 150 volts each have their negative poles all connected to the shield. The switches S_2, S_6 and S_8 permit changing from a condenser to a transformer circuit. S_7 is a shunting switch to charge the output condenser. S_3 changes the set from a two to a three stage amplifier. S_4 and S_5 change the set from a two stage to a one stage amplifier. S_1 changes the optimum input impedance for the input transformer from 20 to 120,000 ohms.

the last tube is shunted across the 200,000 ohm resistance through a 14 microfarad condenser and the galvanometer string. The condenser has a resistance of 500 million ohms. In a few experiments a 34 microfarad condenser was used. As an alternative it is possible by the use of the double throw switches, S_6 and S_7, to connect the output of the last tube to the primary and the string to the secondary of a transformer. The optimum output impedence of this transformer is 500 ohms, and the input impedence matches that of the tube.

The vacuum tubes are the Western Electric type V. They are designed for use with a filament current of 1.3 amperes, a grid voltage of -1.5, a normal plate voltage of 100, and a maximum input voltage of 2. The value of the amplification constant μ is 28. The plate current under the conditions of our circuit is 0.45 milliampere. The d.c. plate-filament resistance is therefore 155,000 ohms and the a.c. resistance 77,500 ohms.

The actual wiring of the amplifier is nearly like that of the diagram. The whole is within the indicated shield consisting of a wooden box with a top and bottom of $\frac{1}{8}$ inch steel and a lining around the sides of $\frac{1}{16}$ inch lead. The box is padded on the inside with $\frac{3}{4}$ inch wool felt. The three tubes are screwed to a pine board and the latter wedged about with rubber bath sponges. Switch S_3 makes it easy to cut out one tube and obtain a two stage amplifier using the two outside tubes. In practice we never used more than the two tubes. Switches S_4 and S_5 were inserted subsequently in order to use one tube only. The rather heavy amplifier box with hinged lid and outside connecting switches for the various batteries is supported on rubber stoppers and placed inside a galvanized iron box not indicated on the diagram. This second shield may have been unnecessary and it would probably have been better to have improved the mechanical insulation of the tubes and placed each of them in a separate steel compartment with sufficient room to make the wiring straighter and simpler.

The input wire was protected by a grounded lead conduit with the exception of a short wire connecting it to the zinc electrode and of a double throw switch inserted for an alternative direct connection to the galvanometer. The amplifier was located on the opposite side of the room from the galvanometer, a distance of 14 feet, to protect it from the currents of the lantern, galvanometer magnet and camera motor. The camera motor and attachments were shielded with sheet-metal as was also the tuning fork, except for a small aperture for the reed attached to one of the prongs. The output wires were carried unshielded across the room to the galvanometer. When using the transformer the protecting resistances in the Leeds and Northrup box, designed by Williams for use with the string galvanometer, afforded sufficient protection. When the output condenser circuit was used a shunt switch S_8 allowed the condenser to be charged before connection was made to the galvanometer box through switch S_7. When connection is made to the string the added circuit must be charged through the string. It was, therefore, found advisable to connect an Ayrton

universal shunt across the string, as this affords a better protection against unforeseen potentials in the output, since it is necessary to start with a 0.0001 part of the output through the string and proceed through the fractions of 0.001, 0.01, 0.1 to the total output current. The leak of the condensers was so small that with a tense string compensation was unnecessary. With a loose string the leak was conveniently compensated by employing a second galvanometer box across the output terminals of the first.

It is of course necessary to have all the input connections and amplifier switches in position before starting to throw in the galvanometer. When using the condenser circuit disturbing the electrodes or touching the input will usually break the string if the galvanometer is connected. With the transformer circuit precautions of this sort are not so necessary.

All amplifiers, no matter how carefully designed have a tendency to sing, that is, to produce sustained oscillations of one or more frequencies. Most of our time has been taken up in minimizing this effect. Although we have been quite successful in this respect we are not yet certain just what factors enter into the production of a quiet system. It is necessary to have good electrical, mechanical and sound shielding of the apparatus. The wiring must be simple and avoid as much as possible inductance between the circuits of the different tubes. The ground circuit must be heavy, the leads from it must not be too widely distributed and the galvanometer ground should also come to the same common point. We feel that our electrical and sound shielding are adequate but that our mechanical shielding needs to be improved. The tubes themselves tend to sing less after they have been continuously in use for a long period of time.

The apparatus was tested for amplification. By this is meant the ratio of the deflection of the string of the galvanometer with the amplifier to that occurring without it. The magnitude of the effective amplification is a function of the resistance of the preparation. This is due to the fact that the deflection of the string depends upon the current going through it and, therefore, for a given potential is small when the resistance in the preparation is large; while on the other hand the vacuum tube tends to act as an electrometer. This follows because the current released by the tube is proportional to the alternating current voltage drop across the grid resistance. If e_n is the voltage developed by a nerve of resistance r_n, the voltage drop e_g across the 2 million ohm grid resistance is

$$e_g = e_n \frac{2{,}000{,}000}{r_n + 2{,}000{,}000}$$

The resistance of the nerve being small compared with 2 million ohms, changes in the resistance of the nerve produce but small changes in the voltage across the grid resistance.

When the deflection of a 3820 ohm string produced by 0.84 m. v. obtained from a potentiometer was compared with the deflection obtained when the same potential was impressed upon the grid of a one tube amplifier, the amplification was found to be only 8.7 per cent. When however 70,000 ohms, a resistance found in two of our nerve preparations, were added in the connection to the string or to the grid, the effective amplification became 27 times for one tube and 559 times for two tubes, an advantage of two tubes over one tube of 20.7 times.

Low operating efficiency, more apparent in the first instance, is due to power loss in the output and input circuits. The power developed in a string of a resistance r_o by a voltage e_g impressed upon the grid of a tube having a plate resistance r_p and an amplification constant μ is

$$\frac{\mu^2 e_g^2 r_o}{(r_p + Z_o)^2}$$ (Van der Bijl art. 63)

Z_o (defined below) can in the present argument be considered approximately equal to r_o. As the value of r_o is small compared with r_p the development of power in the string falls considerably below a maximum. When a source of potential e_n of internal resistance r_n is connected directly to the string the power developed in the string is

$$\frac{e_n^2 r_o}{(r_n + r_o)^2}$$

We have seen that e_n is nearly equal to e_g and therefore if r_n is small compared with r_o, as is the case when leading directly from the potentiometer, this expression may be as large as or larger than the one above. The tube acts as a generator of potential μe_g with a high internal resistance r_p. It operates into the galvanometer string which has a relatively low resistance r_o. For the power expended in the string to be a maximum r_o should equal r_p. Increased output efficiency could be obtained by matching the string impedance to the tube impedance through a transformer. Similarly matching the resistance of the source of potential to the grid resistance through an input transformer would step up the input voltage and increase efficiency.

It is usually for other reasons undesirable to use transformers. It is better to gain amplification by stepping up the input voltage e_n with additional tubes and it is by this means that the desired effective amplification from a tissue of any resistance may be obtained. The condenser output connection has been found to be generally more practicable than the transformer connection. Curves obtained with the latter must be integrated once and if there is also an input transformer twice, in order to obtain the form of the input wave. The transformer connection is nevertheless of value under some circumstances. It can be made to amplify more efficiently and it is as free from noise.

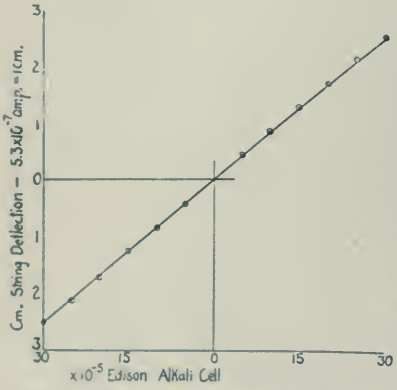

Fig 3. The points are a plot of the string deflection against known grid potentials when using two tubes and a 14 microfarad output condenser.

The conditions for distortionless amplification are that the impressed grid potential shall not be large enough to cause the grid to absorb current, that the dynamic characteristic of the amplifier shall be linear and finally that the amplification shall be independent of the frequency. The input potentials with which we are dealing are so small that the first condition does not enter into consideration. The second condition makes it necessary that the deflection of the string shall be a linear function of the input potential, as far as the increasing tensions of the string, on deflection, allow this to be true, and that the angle of external impedance shall be nearly zero. As a two tube condenser circuit was found to be the most useful we have plotted the deflections produced by such a system against the range of input potentials that will cause deflections of a tense string which do not exceed the limits of 6 cm. paper. In this range, which includes most of our working range, the amplification is practically a constant as the points all come very close to a straight line (fig. 3).

The impedance of a circuit is determined by its resistance, inductance and capacity and is the denominator of the expression

$$I = \frac{E}{\sqrt{R^2 + \left(2\pi fL - \frac{1}{2\pi fC}\right)^2}}$$

where I is the current in amperes, E is the potential in volts, R is the resistance in ohms, f is the frequency, L is the inductance in henries and C is the capacity in farads. The expression in the parenthesis is known as the reactance. The angle of impedance is the arc tangent of the reactance divided by the resistance.

The inductances of the amplifier circuit are all very small and may be neglected. The important reactances occur in the output and coupling condensers. The external portion of the alternating current circuit of the last tube is essentially the 14 microfarad condenser and the 3820 ohm string. The external portion of the circuit of the first

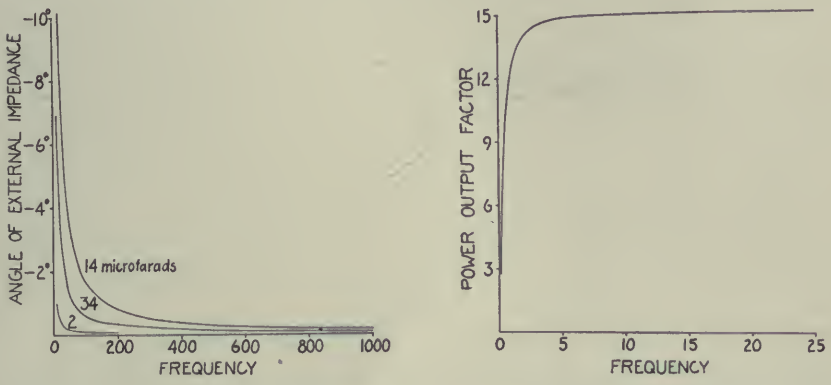

Fig. 4. *a:* The curves are the angles of impedance $\frac{x_o}{r_o}$, for the intertube circuit consisting of a two microfarad condenser and a 500,000 ohm resistance and for output circuits consisting of a 14 or a 34 microfarad condenser and a 3820 ohm string.

b: The curve is a plot of that fraction of the expression for power expended in the string which varies with the frequency of the impressed grid potential.

tube consists of a 2 microfarad condenser and a 500,000 ohm resistance. The angles of external impedance of these two circuits are plotted in figure 4 a. Both angles are quite small, especially for frequencies above one hundred. The total angle of external impedance is therefore small and the dynamic characteristic of the amplifier is substantially linear.

The third condition is satisfied if the impedances of the circuit do not vary sufficiently with the frequency of the input potential to distort the power amplification. The reactance of the coupling condenser is so small that it is only necessary to consider the effect of the condenser in the output circuit. In order to obtain a simple expression for the

power developed in the output circuit the last tube may be considered as being a generator of an alternating potential E, having a high internal resistance r_p. Omitting the small shunting effect of the 200,000 ohm resistance the impedance of the external circuit is

$$Z_o = \sqrt{r_o^2 + \left(\frac{1}{2\pi f C}\right)^2}$$

where r_o is the resistance of the string and C is the capacity of the output condenser. The current in the circuit is

$$\frac{E}{r_p + Z_o}$$

and the potential drop across the string is

$$\frac{E r_o}{r_p + Z_o}$$

The power expended in the string is the product of these two and equals

$$\frac{E^2 r_o}{(r_p + Z_o)^2}$$

The denominator only of this expression contains the factor which varies with the frequency and therefore the value of the expression

$$\frac{1}{(r_p + Z_o)^2}$$

for different frequencies is plotted in figure 4b. The amplification reaches 99 per cent of its maximum as soon as the frequency reaches seven per second and is thus practically independent of the frequency for frequencies above this value. The amplifier thus satisfies all the conditions for essentially distortionless transmission for frequencies above one hundred.

It is necessary however to consider the transient phenomena which occur in the circuit. If a potential $e = E\cos(\omega t - \theta_o)$ be impressed upon a circuit containing resistance, inductance and capacity in series, the expression for the current, i, in the circuit consists of a permanent and a transient term, namely, $i = \frac{E}{Z_o}\cos(\omega t - \theta_o - \gamma)$ + an exponential

term, (vid. Steinmetz (16)), where $\omega = 2\pi f$, t is the time, θ_o is the phase angle determining the instantaneous value of the impressed potential at the start, and γ is the impedance angle. The exponential or transient term depends not only upon the constants of the circuit but upon the nature of the impressed electromotive force and its phase at the moment of starting. In practice under our conditions the instantaneous starting potential is always zero, $\theta_o = 90°$. For the constants of our circuit and an alternating current of a frequency of one hundred per second starting when the instantaneous potential is zero, the transient term is a logarithmic curve, small in comparison with the maximum amplitude of the permanent term. During the period that the transient term is in operation the current records will oscillate about this curve as the line of zero potential. In case the impressed oscillatory potential is unidirectional, considerable energy is stored in the capacity before a permanent condition is reached and the transient term is a logarithmic curve whose initial value, as we shall see later, is large. Its slope is gradual and therefore only the low frequency harmonics are appreciably distorted during the period of several seconds in which the term is effective.

The current described by the permanent term, $\frac{E}{Z_o}\cos(\omega t - \theta_o - \gamma)$ has only a phase distortion which depends upon the values of f involved in the analysis of the impressed potential function. If the values of γ are small there will be but little phase distortion. Assuming that the negative variation in nerve lasts 5 thousandths of a second, the curve of potential can be analyzed into a fundamental of a frequency of one hundred per second and harmonics. The phase deviations of the fundamental and the ninth harmonic are respectively only 1.7° and 0.17° with a 14 microfarad condenser and 0.56° and 0.08° with a 34 microfarad condenser. The phase differences between the fundamental and the ninth harmonic are therefore 1.53° and 0.48° in the two cases.

There is a graphic way of looking at the distortion by the condenser circuit. When the grid potential is changed the tube resistance rises or falls and the potential of the condenser terminal must correspondingly rise or fall. A current will accordingly flow through the galva nometer between the condenser plates. The condenser reaches its equilibrium potential slowly if it has a large capacity to be charged through a high resistance. The charging (or discharging) current rapidly reaches its maximum under Ohm's law, then falls away logarithmically

as the condenser is charged (fig. 5). The falling off of the current in 0.01 second can not be measured with any accuracy but as the rate of change is logarithmic, it can be calculated for any point from any other two points. Such a calculation shows that the falling off is about 0.84 per cent in 0.01 second and not sufficient to produce an appreciable distortion in currents of short duration. In fact, changes as slow as the currents obtained by Einthoven from the vagus (17) are readily recorded, though with relatively more distortion. Figure 6 is included to show the form of the amplified electrocardiogram and its freedom from other vibrations.

Fig. 5

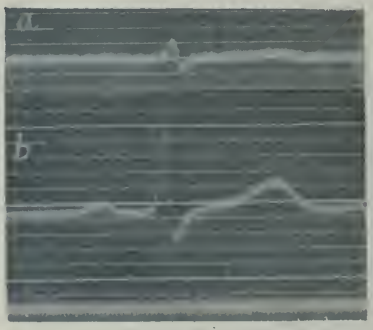

Fig. 6

Fig. 5. The deflection produced by a constant potential of 20 millivolts in a 3030 ohm string when in series with a 20 microfarad condenser and a 77,000 ohm resistance. The added line indicates the rise in potential of the condenser plate as the condenser fills. Time, $\frac{1}{5}$ second.

Fig. 6. Electrocardiograms taken from the right and left forearms by means of non-polarizable electrodes of the $ZnSO_4$ type having an area of contact of about 1 sq. cm. Time, $\frac{1}{10}$ second.

a, Connections made directly to the galvanometer.

b, Connections made to the galvanometer through a one tube amplifier. The conditions are otherwise the same as in *a*.

EXPERIMENTAL APPLICATION OF THE AMPLIFIER

The recording of the action current of the right phrenic of the dog was first tried with *amplification by one tube*. A typical result is shown in figure 7. The unamplified action current, *a*, may be compared with the amplified, *b*. The latter is selected from a series in which the string was progressively loosened to obtain the maximum oscillation, and presents a picture which requires some explanation.

The demarcation current in the nerve acts like any other battery producing a grid bias and contributes its part to the determination of the constant potential of the output condenser. The action currents produce deflections of the string downward. In addition to the individual deflections there is a sustained downward deflection of the string. This sustained deflection increases during the first portion of the inspiratory period, but the increase stops before the end of the period and very close to the end decreases rapidly, the string undergoing a rapid deflection upward to a point well beyond its equilibrium position.

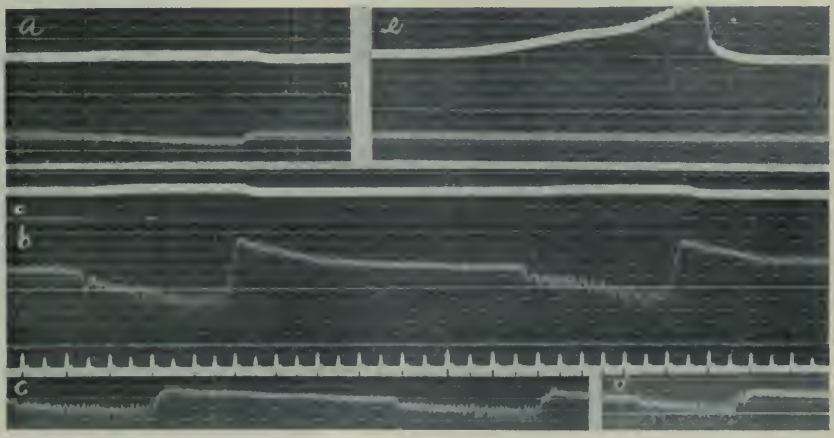

Fig. 7. Action currents from the right phrenic of the dog. Leads from the side and cut end of the nerve.

a: No amplification. Time same as in *b*, $\frac{1}{5}$ second. Upper line pneumograph, inspiration upward.

b: Amplification with one tube. Proximal electrode on the nerve connected to the grid; when it is negative the deflection is downward.

c, d: Same as *b* with string at greater tensions. The pneumograph and time tracings have been cut off.

e: Same as *b* except that nerve has been killed proximally to the proximal electrode. Time in *c*, *d*, *e* the same as in *b*.

This picture may be explained as the sum of the effects of the galvanometer, the increasing size of the individual nerve current waves and the condenser. It may be reproduced experimentally by recording a rapidly interrupted current from a potentiometer under similar conditions.

If unidirectional and equal changes in potential are impressed upon a tense string at intervals of the order of 1 one-hundredth of a second

the string will approximately follow the potentials so impressed and will oscillate between the zero axis and the maximum deflection about a mean position above the axis. This mean position is determined by the mean value of the potential function and is given by the definite integral of the potential in the time interval divided by that interval. If, on the other hand, the impressed changes are increasing in magnitude the line about which the string oscillates is no longer horizontal but is inclined from the axis. If under these conditions the string be loosened so that it no longer follows completely the rapid fluctuations in potential it will still oscillate about a mean position which is the mean as understood above. The distance of this mean from the axis will be greater because of the greater sensitivity of the string. The size of the individual oscillations, while it may be greater than obtained with a tense string, becomes a relatively smaller fraction of the deflection produced by a constant current of the same potential and therefore a smaller fraction of the curve of true potential.

In *a* and *b* of figure 7 the increasing distance from the zero axis of the line about which the string is oscillating is the result of an increase in size of the individual waves. In the latter part of an inspiration, in the record of the amplified current, it can be seen that in spite of the fact that the size of the individual waves is increasing the string begins to shift slowly upward. At this point the effect of the condenser, no longer masked by the increasing downward deflection, has become apparent.

The form of a complete condenser charging current is shown in figure 5. The quantity of current which has entered the condenser is measured by the area under the curve. On removal of the potential from the condenser terminals a curve of the same shape and size is obtained but it is in the opposite direction. If the charge of the condenser is not complete it is equal to the area under the portion of the curve which is completed and the initial current dissipating this charge is determined by the potential which the condenser has acquired and is measured by the difference between the initial and final charging currents. The area between the discharging current and the axis is equal to the area beneath the charging current. The time for complete discharge, being a matter of several seconds, is long compared with discharge intervals of less than 1 one-hundredth of a second with which we are dealing. The condenser thus receives with each wave a greater charge than it loses in the interval. The condenser accumulates a charge and a potential which results in interval discharge currents of increasing

magnitude. Equilibrium is established when the loss of charge in the interval becomes equal to the charge gained. The effect of the condenser is therefore to lower the line about which the string is understood to be oscillating until the line coincides with the zero axis.

In figure 7, *b*, the negative potential produces a downward deflection and the accumulating charge of the condenser an upward shift which is overbalanced by the increasing size of the waves during the first part of the inspiratory period. At the end of the period the rapid decrease in size of the waves results in a rather abrupt upward shift of the string due in part to a decrease in the mean about which the galvanometer string is oscillating and in part to an increase in the discharging phase of the condenser. On the cessation of the waves the string moves to a position above the zero axis commensurate with the potential which the condenser has accumulated during the interval. The discharging current has the usual logarithmic form.

The true potential waves of negative variation would be represented by an oscillation between a logarithmic curve connecting the initial zero of the string and the point of maximum discharge current and another curve determined by this curve and the line of mean oscillation.

When *amplification with two tubes* was tried it was found to be far superior to that obtained with one. Such a result is recorded in figure 8. This record was made with a string tightened to the border of periodicity. The tight string follows the individual waves and therefore the bases of the waves return to a line indicating zero potential. The progressive movement of the bases of the waves upward due to an accumulating charge on the condenser can now be seen without the complicating effect of the slow galvanometer string. Destruction of physiological continuity by heating or crushing a segment proximal to the proximal electrode caused all signs of action currents during inspiration to disappear.

A direct comparison was now made of the relative values of one and two tubes. The leads were, in this case both from the side of the nerve, but the connection at the diaphragm had been severed so that the nerve could be looped through the electrodes in the manner previously described. With the string tightened to the border of periodicity the sensibility was still such when two tubes were used that the oscillations exceeded the limits of the 6 cm. paper (fig. 9, *c*). A small amount of "noise" is visible in the respiratory pause but is insufficient to seriously interfere with the record.

Fig. 8. The action currents of the left phrenic of the dog obtained with the aid of two tubes and an output condenser of 34 microfarads capacity. The distal electrode on the nerve is connected to the grid of the first tube. Negativity of the proximal electrode is indicated by a deflection downward. Time $\frac{1}{100}$ second, the same in both records. Calibration of the string 5.5 $\times$ 10^{-7} amperes = 1 cm. Upper line pneumograph, inspiration upward. The upper record is the same as the lower except that the nerve had been killed proximally to the electrodes.

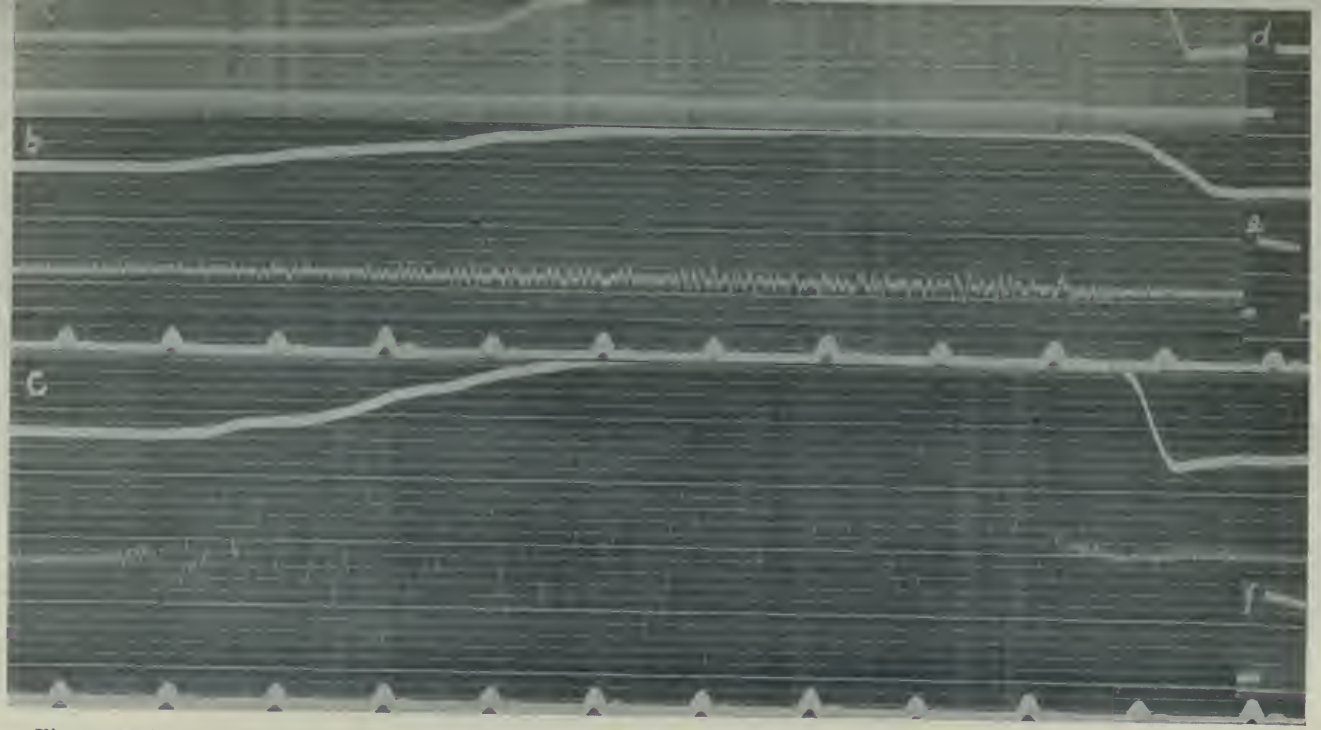

Fig. 9. Action current records from the right phrenic of a dog taken in as rapid succession as possible.

a: Unamplified. The leads are from the side of the nerve to the cut end; 9.3×10^{-8} amperes = 1 cm. The line above the string shadow in each record is the pneumograph shadow, inspiration is upward. The time record has been cut off, it is the same as in *b* and *c*, in $\frac{1}{5}$ second.

b: Amplification with one tube; 9.3×10^{-8} amperes = 1 cm. The leads are both from the side of the nerve. Resistance of nerve plus electrodes 110,000 ohms. Negativity of the proximal electrode produces a deflection downward.

c: Amplification with two tubes; 5.2×10^{-7} amperes = 1 cm. Leads as in *b*. Negativity of the proximal electrode produces a deflection upward.

d, e, f: Data for calculation of the amplification. String at a constant tension; 5.5×10^{-7} amperes = 1 cm. String resistance 3820 ohms.

d: No amplification; 84 millivolts across 110,000 ohms and the string.

e: One tube; 2.52 millivolts with 110,000 ohms in series in the grid circuit.

f: Two tubes; 0.168 millivolt with 110,000 ohms in series in the grid circuit.

This record may now be compared with *b* in figure 9, which was made from the same leads using one tube. The string was now looser, having been let out empirically to obtain the maximum oscillation. It is readily seen that under these conditions the distinctness of the individual oscillations has been sacrificed in the interest of amplitude. As the potential changes when this lead is used are not unidirectional the slow changes in the position of the string which have been described above are negligible. Without amplification no visible oscillation appeared in the string when it was used at a tension as great as that employed in recording the output of two tubes, therefore the string was not photographed. Figure 9, *a*, shows an unamplified record from the nerve when the distal lead was changed from the side to the cut end and the tension was changed to an optimum. As the leads from the side of the nerve were well separated the resistance of the nerve was large, 110,000 ohms. The effective amplification was therefore also large, the factor being 36.5 for one tube and 624 for two tubes.

OBSERVATIONS ON THE ACTION CURRENTS OF THE PHRENIC NERVE

The waves of negativity appear in the record at the beginning of an inspiration. They increase gradually in size during the early part of the cycle and attain a maximum which they hold to close to the end of the cycle when they terminate much less gradually, sometimes quite abruptly. An envelope of the crests would therefore have a shape roughly corresponding to the pneumograph shadow. This indicates that the gradual even contraction of the diaphragm is occasioned by the calling into activity of more and more nerve fibers as inspiration deepens. On the other hand the more abrupt falling off of the phrenic action current corresponds with the well-known fact that the expiratory limb of the respiratory tracing is much steeper, expiration occurring passively as the innervation to the muscle is withdrawn. In none of our records have we seen any evidence of innervation of the diaphragm during the intervals between inspirations.

While variation of the average size of the waves occurs in different parts of the respiratory cycle, the average rate seems to be constant throughout. In some records the last five or six waves appear at intervals smaller than the average but this is not a constant occurrence. The action current has the appearance that would be produced by discharges from the cord closely resembling volleys. The intervals between the volleys, while they show considerable regularity, are by

no means constant even when the waves are most smooth and regular. On the other hand the variation in the rate at which the waves appear is not as great as their difference in amplitude.

The waves are definite enough throughout an inspiration so that their total number can be determined with only a small percentage of error. They were counted in a number of experiments and the results are listed in the following table.

TABLE I

	NUMBER OF WAVES IN ONE INSPIRATION	DURATION OF INSPIRATION	RATE PER SECOND
		seconds	
Dog A	138	1.59	87
	115	1.29	89
	120	1.30	92
	108	1.24	87
Dog B Consecutive inspirations	176	1.90	93
	184	1.89	97
	178	1.88	95
	175	1.80	97
	159	1.75	91
Dog C	145	1.43	101
	144	1.39	103
	149	1.40	106
	113	1.16	97
	126	1.21	104
Dog D	78	1.105	72
	90	1.21	74
	80	1.095	73

No generalization as to rate is justifiable from these data. They only hold for the conditions of the experiment. The temperature of the animals was not controlled. While precautions were taken to prevent cooling, the temperatures of the animals were undoubtedly several degrees centigrade subnormal. The rates recorded in the different experiments vary from 87 to 106 per second. In one animal in poor condition the rates counted were 71 to 74. The normal innervation rate would probably be higher, not lower.

In a number of records made on rapidly moving paper the intervals between the consecutive waves throughout the period of activity were carefully measured in the search for some evidence of system in the

irregularity. For instance in one inspiration of 138 waves, the average separation was 10.95 σ; the variation was from 8.8 σ to 17.9 σ. Excluding 22 of the extreme intervals, the variation from the average was from minus 13 per cent to plus 19.6 per cent. In the most regular record obtained, where the rate of discharge was slower (71 per second), in 61 consecutive volleys of a series of 78, 77 per cent were within 10 per cent of the average. But no evidence of any recurrence in the irregularity was visible.

In addition to difference in size and distance apart, there is also a variation in the shape of the waves. To account for the latter the possibility of differences in phase and of differences in period in the different fibers must be considered. In referring to a difference in phase it is intended to designate a condition in which the nerve fibers are all alike, the velocity of propagation of the nerve impulse and therefore the period during which the electrode is affected by the negative variation being the same in all, but in which, due to the fact that the disturbance is not started in all the fibers at the same instant, it does not reach a given level in the nerve in all fibers simultaneously. It is clear, however, that there cannot be many different phases or the wavelets would either be very large in number or they would fuse producing a waveless record. Furthermore the fibers cannot be mutually far out of phase or it would be unlikely that groups of large waves free from superimposed waves would be obtained. At times all the fibers are very close to being in phase because the individual action currents have the well-known appearance of the action current obtained in nerve or muscle from a single induction shock. This can be seen in figure 8, in which it should be borne in mind that negativity of the proximal electrode produces a downward deflection. To bring this fact out more clearly, a record, taken on paper moving 25 cm. per second and from a nerve in which the rate of discharge was slow (71 per second), was redrawn. The points used for plotting were taken with the aid of a binocular magnifier. In the graph, the ordinate (deflection of the string) was unaltered in size, but the abscissa (time) was multiplied by 5. This gives the appearance which the record would have if the photographic paper had been run 5 times as fast. The appearance of both a monophasic and a diphasic lead from the same nerve is thus shown in figure 10.

If the impulses are discharged into the nerve at regular intervals one would expect that if they are merely out of phase there would be some tendency of the curves to repeat themselves. This would also be true if the component waves have a different period. Differences

in period could arise from the fact that the duration of the negativity under the electrode is not the same in all elements of the nerve, a condition arising from different velocities of conduction as a result either of physiological differences in the constituent fibers or of variation in exposure to injury. In the latter case there would be a tendency toward beat production, and of this the records show only the faintest tendency. We must, therefore, consider that the intervals at which the spinal cord discharges impulses into the nerve are within limits irregularly variable, that the number of fibers involved in the individual volleys varies and that the disturbance is started at times nearly simultaneously in all the fibers; at other times there is a slight difference in phase which may become so large that accessory waves may appear on the main wave.

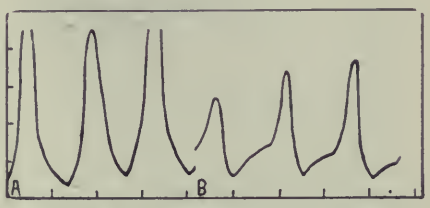

Fig. 10. Action currents of the phrenic nerve plotted from a record. *A*, monophasic; *B*, diphasic, from the same nerve. Ordinates, same as in record, 1 division = 1 cm. Abscissae multiplied by 5, 1 division = $\frac{1}{100}$ second.

Considerable interest lies in an estimation of the rate of discharge through an individual fiber. The difficulty in determining this rate lies in the waves that may be considered either as a portion of one volley somewhat out of phase or of another volley involving perhaps a small number of fibers. The error in counting on account of such waves is fortunately not as large as might be anticipated. The fair accuracy of the counts recorded in table 1 appears in the fact that for a wave in the nerve a corresponding one can be obtained simultaneously in the muscle. This was done with two galvanometers arranged tandem on the optical bench, the image of the string of the first galvanometer being focused by the second in the same plane as the image of its own string.

One galvanometer was connected to the anterior part of the right side of the diaphragm, the two electrodes lying on a radius from the motor point and in such a way that a diphasic action current was obtained with the first phase upward when the proximal portion of the muscle was negative. Two leads were made from the side of the right phrenic to the amplifier. The proximal electrode was connected to the grid of the first tube. Two tubes were used, but in spite of as complete isolation of the nerve as possible only one-tenth of the output could be recorded on account of extraneous currents, especially the

electrocardiogram. An idea of the effect of the latter may be obtained from the fact that in a record in which the whole output was recorded the "P" wave was 4 cm. high and the "R" wave was 7.8 cm.

Figure 11 is a record of the action currents of the right phrenic nerve and the right side of the diaphragm, taken simultaneously during one inspiration. Lines have been drawn from each wave on the phrenic nerve record to the corresponding one on the diaphragm record. The slope of the lines is apparent; the crest of a wave in the muscle action current comes about 9 σ after the crest of the corresponding wave in the nerve action current. This measurement does not allow for the

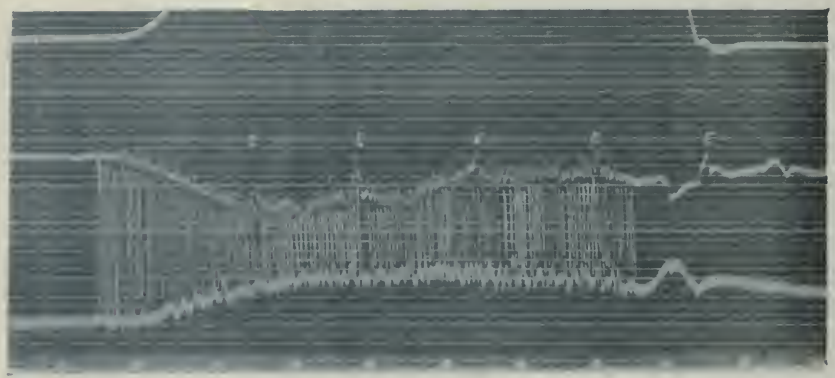

Fig. 11. Upper line: pneumograph, inspiration upward. Upper galvanometer tracing: leads from the side of the right phrenic nerve. Two tubes; $\frac{1}{16}$ of the output is recorded; 5.2×10^{-7} amperes = 1 cm. Deflection upward when proximal electrode is negative. Points marked *E*, electrocardiogram.

Lower galvanometer tracing: Action currents of the right side of the diaphragm recorded simultaneously with the nerve action currents. Negativity of the proximal electrode produces a deflection upward. Time in $\frac{1}{5}$ second.

phase lag of the loose string, recording the muscle action current, behind the tense string recording the nerve. Throughout the whole record corresponding waves can be identified. Moreover, there is a close correspondence in the size of the waves in the two records. The action current waves of the muscle are unevenly spaced but in every case it will be found that in the nerve record, preceding them by 9 σ, there will be waves with corresponding intervals. This is more clearly brought out in faster records.

An interesting observation is the symmetry of the action current records on the two sides. Records were made from the right phrenic

nerve, leading from the cut distal end and the side. This allowed the recording of the whole output from two tubes. Simultaneous with this was recorded the action currents of the opposite side of the diaphragm. The uneven spacing of the waves appears in both records but the intervals are the same on the two sides. While more data would be desirable on this point, the evidence indicates that the intervals at which the impulses are discharged from the cord into the nerves of the two sides is controlled from the same common point.

In comparing the nerve action current with the muscle action current recorded with a loose string it is often apparent that a very small wave in the muscle record, appearing sometimes as a notch on the side of a larger wave, has as its counterpart in the nerve record a definite wave. It must, therefore, be counted as a separate volley and not as a portion of some other volley out of phase, as is sometimes done. Piper's conclusions have been criticised in that, while the individual action currents in the muscle may be determined by corresponding disturbances in the nerve, the rate at which they appear is not fifty. We are not prepared to enter into this discussion at length. The rate of discharge into the phrenic nerve, even under the adverse experimental conditions, was twice this. The rate of the waves obtained from the forearm must depend on the technique employed and the basis on which they are counted. In our experience, when leads were made with small electrodes of one square centimeter surface from two points distal to the muscle equator, and recorded, after amplification, with a tense string, the rate was definitely one hundred or more.

SUMMARY

An apparatus is described by which physiological action currents as they appear in the phrenic nerve may be satisfactorily recorded.

This is done by a two stage three electrode vacuum tube cascade, resistance coupled, and connected to a string galvanometer through a large condenser.

The following points are brought out by the action current records:

1. Inspiration is brought about through discharge into the diaphragm of 78 to 178 volleys of impulses at the rate of from 71 to 106 per second, under varying conditions of the experiments.

2. The waves vary in size, suggesting variation in the number of nerve fibers involved. The average size of the waves is larger near the end of inspiration.

3. At slow rates of discharge the typical form of an artificially produced action current is apparent. Variations in the form occur and are probably caused by the fact that the discharges by the cord into the different fibers are slightly out of phase.

4. The intervals between the volleys are irregular.

5. Simultaneous records taken from the phrenic nerve and diaphragm show that every wave in the muscle is produced by a corresponding one in the nerve. The waves correspond in size and in the intervals of separation in the two records.

6. The similarity in the spacing and size of the volleys on the left and right sides suggests that the discharges from the cord into the two phrenic nerves are controlled from some common point.

We wish to thank Mr. R. E. Bitner and Mr. I. B. Crandall for helpful advice.

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THE OUTPUT OF THE HEART IN DOGS

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Received for publication April 18, 1921

About twenty-five years ago (1) I described a method of measuring the amount of blood passing through the lungs and of following changes in that amount A considerable number of measurements on dogs anesthetised with morphine and ether, or with morphine and ACE mixture were published. The principle of the method is that a solution of a substance which can be easily recognized and quantitatively estimated in the blood is permitted to flow for a definite time at an approximately uniform rate into the heart. The injected substance mingles with the blood and passes out with it into the circulation. At a convenient point of the vascular system (usually from a branch of the femoral artery) a sample of blood is drawn off just before the injection and another during the passage of the substance; and the quantity of the solution which must be added to a given volume of the first sample, in order that it may contain as much of the injected substance as the second sample, is determined. This determination gives us the means of estimating the extent to which the injected solution has been mixed with blood in the heart, and thus, knowing the quantity of the solution which has run into the heart, we can calculate the output in the given time. I used a solution of sodium chloride for injection. The arrival of the mixture of blood and solution at the point of collection is easily detected by exposing a portion of the opposite artery at the same level as the collecting cannula, placing it on electrodes connected with a Wheatstone's bridge, and balancing the bridge till the sound in the telephone is a minimum. As soon as the blood mixture reaches the point of collection the balance of the bridge is upset, the sound increases more or less rapidly (according to the velocity of the blood and the quantity and strength of the salt solution injected) to a maximum, at which it remains steady till the greater part of the column of altered blood has passed, and then declines again more or less rapidly. When

the injection is made into the left ventricle, which is preferable for most purposes, but not indispensable with salt solution, the interval between the commencement of injection and the beginning of the sound is, of course, much shorter than when the solution is injected into the right heart. Also, the maximum is more rapidly attained and the sound disappears again more abruptly. The question whether the salt solution is uniformly mixed with the blood in the heart, when the injection is uniform and the inflow of blood into the heart steady, was examined and answered in the affirmative. Successive samples, collected while the blood mixture was passing and while the sound remained steady, had approximately the same conductivity.

In the majority of the observations the salt solution was allowed to run into the heart for so long a time (10, 12 or even occasionally 15 seconds, depending on the velocity of the blood stream and the size of the animal) that the *rate* of injection could be accurately estimated. The time for which a uniformly altered column of blood continues to pass the point of collection is, of course, increased with the time of injection. A sample collected during the time when the sound remains steady, or a considerable portion of that time, will obviously show the increase of conductivity which the salt solution entering the heart at the given rate is maintaining in all the blood passing through the heart. Thus, if m cc. of salt solution must be added to 1 cc. of the normal sample to make its conductivity equal to that of the sample collected during passage of the blood mixture, and Q cc. of solution in T seconds, i.e., $\frac{Q}{T}$ cc. of solution per second was injected into the heart, then $V = Q\frac{60}{mT}$, where V is the volume of blood passing through the heart in a minute (procedure I).

Besides reckoning the output from the maximum steady increase in concentration of the injected substance in the collected sample and the rate of injection, it can be estimated in another way, which was also employed by me. The blood is collected for the whole period during which the mixture is passing the collecting cannula, including the preliminary period when the concentration is increasing to the steady maximum and the final period when it is diminishing again. The amount of salt solution necessary to raise the conductivity of the normal sample to that of the sample collected in this way during passage of the mixture will clearly be less than that required for a sample collected only at the maximum concentration, and the greater the "tailing off"

of the column of blood, the smaller will be the increase in the specific conductivity of the whole sample. This does not affect the result, however, provided that instead of the rate of injection, we take the total quantity of solution injected and consider that this quantity has mingled with all the blood passing through the heart, not during the time of injection but during the time of collection of a complete sample of the blood mixture. That this assumption is correct, so long as the output of the heart remains steady, is easily seen if we consider that the first and last parts of the column represent mixtures in which a given mass of injected salt has been distributed over a larger quantity of blood coming to and leaving the heart than when the steady state has been reached. The greater amount of blood with which the salt solution has mingled, calculated from the smaller average increase of conductivity of the entire sample, will, however, be precisely balanced by the increased time over which this greater quantity of blood is being discharged. The same is true even if collection of blood is begun at the moment when the injection commences or at any time between that moment and the arrival of the first portion of the mixture at the point of collection. For the normal blood added to the sample will dilute it in proportion to the extra time of collection. Thus, if Q is the number of cubic centimeters of salt solution injected, t the time of collection of the sample of the blood mixture in seconds, and m the quantity of salt solution (expressed as a fraction of a cubic centimeter) which must be added to 1 cc. of the normal sample to make its conductivity equal to that of the blood mixture, then $V = Q\frac{60}{mt}$ where V is the number of cubic centimeters of blood passing through the heart per minute (procedure II).

Or, making the formula a little more general, $V = Q\frac{C}{c}\cdot\frac{60}{t}$ where V, Q and t have the same significance as before, while C represents the concentration (number of grams per cubic centimeter) of the injected substance in the solution, and c its concentration in the sample of blood mixture collected. Provided that the rate of collection is uniform, c will not depend upon the rate. For $c = \frac{vc_1t_1 + vc_2t_2 + vc_3t_3 + vc_4t_4}{v(t_1 + t_2 + t_3 + t_4)} = \frac{c_1t_1 + c_2t_2 + c_3t_3 + c_4t_4}{t}$, where v represents the number of cubic centimeters of blood collected per second, c_1 the average concentration of the sample collected in t_1 seconds while the concentration is rising to the maximum, c_2 the concentration of the portion collected in t_2 seconds while it is steady at the maximum, c_3 the average concentration of the portion collected in t_3 seconds while the concentration is declining to zero or to the minimum, and c_4 the concentration of any portion collected in t_4 seconds before or after the blood

mixture has passed the cannula. When a salt not present in the blood is used for injection c_4 is zero and the term c_4t_4 drops out without increasing the numerator. The time t_4, however, increases the denominator, and c is diminished by its inclusion, in the same measure as t (the sum $t_1 + t_2 + t_3 + t_4$) is increased. Precisely the same thing happens, if sodium chloride is injected, c, c_1, etc., then representing the average increase of concentration (or rather, as the method is applied by us, of conductivity) above that of the normal specimen of blood.

In some cases the outputs calculated in the two ways were compared in successive observations on the same animal. For example, in experiment XVI (table 3) the observation at 2:56 was made with collection of a sample with maximum concentration of the salt; 18.6 cc. of salt solution were injected in 7 seconds and collection made from 8 seconds to 15 seconds after the beginning of injection, while the sound was steady at the maximum. The output, calculated from the maximum concentration (procedure I) was 95 cc. per minute per gram of bodyweight. In the next observation, at 3:11, 10.6 cc. of salt solution were injected in 4 seconds, and collection made from 7 seconds to 15 seconds after the beginning of injection, embracing the whole period of passage of the mixture. The output calculated from the average concentration of the whole column (procedure II)was 95 cc. per kilogram of bodyweight. Of course, an exact agreement is only accidental in measuring a quantity like the cardiac output. The total time of injection should be shorter with procedure II than with procedure I, so that collection may be completed before a round of the circulation has been made by any appreciable part of the salt. Henriques (2) has discussed this point in his excellent paper.

In experiment XX (table 4) the observations at 10:46 and 11:04 were on samples collected during the whole time of passage of the blood mixture, the output being calculated by the formula $V = Q\frac{60}{mt}$. In the other observations in this experiment the sample was collected only while the sound was steady at the maximum, the output being calculated by the formula $V = Q\frac{67}{mT}$. The agreement is about as close as is often obtained in two measurements by either of the two procedures.

In 1913 Henriques (2) employed the method, using the second procedure (injection of a definite quantity of a detectable substance and collecting during its whole time of passage). Instead of sodium chloride he injected sodium sulphocyanide. He had no means of knowing when the substance reached the collecting cannula, and therefore employed a very long period of collection, beginning, indeed, as soon as the injection started. His results were obtained under

highly abnormal conditions, his object being to check the conclusion previously come to by Bohr and himself (3), that a substantial fraction of the total combustion in the body takes place in the lungs. For this it was necessary to know how great the coronary blood flow was, in comparison with the total output of the heart, in animals under the conditions of the original experiments. All the branches from the aortic arch (including the innominate and the left subclavian) were tied. The animals received morphine, curare and also leech extract to prevent clotting. Artificial respiration was of course employed. The blood pressure was low in most of the experiments (only 5 are given), and the output of the heart was correspondingly small. But there is no reason to doubt that for the conditions purposely imposed the output was determined with considerable accuracy. For comparison, the results of the 5 experiments, with 3 others (7, 8 and 9) from a second paper (4), are brought together in table 1. It will be seen that in nearly all the experiments there was a progressive decline in the output, accompanied by a fall of blood pressure. I have calculated the standard deviation (σ) and the probable error of the means, which of course is most satisfactory for a series of pretty uniformly distributed values like those in experiment 5 and for a larger number of separate determinations. In this experiment there is a very good agreement between successive determinations. It must be noted

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ERRATUM

Vol. LVII, No. 1, August, 1921

Page 30. Line 8 from bottom, *for* "67" *read* "60."

show generally a very good agreement between separate measurements on the same animal at an interval of several hours or even days, provided that the animal remained quiet during the short time necessary for an observation. Anesthesia is, of course, no guarantee of uniformity in successive observations, made even at short intervals, if the depth of the anesthesia varies or the general condition of the animal (blood pressure, etc.) changes. And it is not difficult to see that under anesthesia the conditions essential for a relative uniformity might alter more rapidly than without anesthesia. However even under the best conditions, an agreement like that between duplicate determinations in a chemical analysis would appeal rather to a chemist than to a physiologist in relation to such a quantity.

I rather insist upon this point because in a recent paper Bock and Buchholtz (5), who have introduced my method for the study of the effect of drugs upon the output, seem to imagine that when they get two successive determinations as close, for instance, as 176 and 179 cc. per kgm. of bodyweight per minute, this is in itself a proof of good technique. Their technique is good, and in one point (the securing of a more uniform injection) they have improved on the original technique. In my opinion, however, they (and also Henriques) have lost something of value in not being able to know the actual time of arrival at the point of collection of the substance injected by them until after the experiment, so

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The experiments of Barcroft and his collaborators (9) on unanesthetised goats show generally a very good agreement between separate measurements on the same animal at an interval of several hours or even days, provided that the animal remained quiet during the short time necessary for an observation. Anesthesia is, of course, no guarantee of uniformity in successive observations, made even at short intervals, if the depth of the anesthesia varies or the general condition of the animal (blood pressure, etc.) changes. And it is not difficult to see that under anesthesia the conditions essential for a relative uniformity might alter more rapidly than without anesthesia. However even under the best conditions, an agreement like that between duplicate determinations in a chemical analysis would appeal rather to a chemist than to a physiologist in relation to such a quantity.

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that they have to guess at the proper time to collect their samples. This would be especially a drawback in studying the output under conditions where considerable changes were occurring in the velocity of the blood stream. Bock and Buchholtz employed the first of the two procedures discussed above, determination of the maximum concentration of the injected substance and the rate at which it is injected. They seem to consider that their experiments were done according to a somewhat different principle than mine. "Ferner richteten wir unsere Versuchsanordnung nach einem etwas anderen Princip ein als Stewart und Henriques." The fact is that Bock and Buchholtz used the principle of our first procedure exactly, while employing a different substance (sodium iodide) for injection, while Henriques employed our second procedure, but with sodium sulphocyanide. Both of these substances are stated to be harmless in the quantities employed, but they cannot possibly be superior to a weak sodium chloride solution in this respect. Both sulphocyanic acid and iodine can be estimated with considerable accuracy, but it is difficult to see how this can be done more accurately than the estimation of differences in the conductivity of blood, such measurements being among the most exact physical determinations. Much the greatest part of the increase of conductivity of the blood when salt solutions in the quantities used in these experiments are mixed with blood is due to the increased sodium chloride content of the plasma, a small part to the increased volume of plasma relatively to corpuscles (6). Both factors act in the same way whether the mixture is effected in the heart or in a test-tube. The determination of the amount of salt solution needed to equalize the conductivity of the normal specimen with that of the specimen collected during passage of the mixture gives, therefore, precisely the same information as the estimation of sulphocyanide or iodide when these salts are injected. It need scarcely be pointed out that the volume of liquid actually ejected by the left ventricle, in the period for which the output is determined, is the volume of blood which enters the ventricle *plus* the volume of the injected liquid. No correction is necessary for this, as it is the volume of blood mingling in the heart with the solution which is measured.

But it is less easy to take account of another factor when numerous injections of solution are made and numerous samples of blood withdrawn in successive observations over a short period of time, namely, the possible changes in the total volume of the circulating blood. Excluding exchange of liquid between the blood and tissues due to alteration of the composition of the blood by the injections (which, of course, cannot generally be completely excluded), no variation in the total blood volume will be caused throughout an experiment if the volume of blood withdrawn is kept equal to the volume of solution introduced. This was approximately the case in our experiments, as shown in table 2. However, with repeated injections the volume of the plasma will increase relatively to that of the corpuscles, unless the volume of liquid injected is small and the time between successive injections sufficiently long to permit the reëstablishment of equilibrium. The viscosity of the blood may therefore be expected to diminish as the experiment proceeds, and in most of our experiments this was clearly seen. We have no definite, uncomplicated observations to show whether a diminished viscosity would of itself cause a greater minute volume.

Bock and Buchholtz have laid stress on the necessity of a uniform rate of injection (when the first procedure is used), while it need not be uniform with the

second. In a general way this is true, but when a sample is collected during the whole period of maximum change in the blood mixture or a great part of it, rapid variations may occur in the rate of injection without detriment so long as the average rate remains constant. Conversely, if the rate of injection is kept uniform, rather rapid variations in the quantity of blood coming to the left ventricle will produce no change in the sample collected according to the first procedure, so long as the average minute volume does not vary.

Henriques states that he was unable to obtain satisfactory results with injection of sodium sulphocyanide into the right side of the heart. It is probable that a portion of the sulphocyanide is retained in the lungs, which would cause the calculated output to be too large. Further, owing to the interposition of the pulmonary circulation the maximum concentration was too slowly reached, and the subsequent decline to the minimum was also slow. We have a far larger mass of data on the measurement of the pulmonary circulation time by the electrical conductivity method (7), with injection of sodium chloride solution. A good maximum is obtained, from which the output can be calculated, with injection of such quantities of salt solution as are permissible, although it is advisable to use larger quantities than those sufficient when injection is made into the left ventricle. As illustrated in experiment XXII (table 4), the results obtained by injection into the right heart are concordant with those found in the same animal with injection into the left heart. But here, of course, the telephone informs the observer of the time of occurrence of maximum and its duration. And if an insufficient amount of salt solution has been injected to give a sharp result, he can modify this at will, while the experiment is going on. This could not be done in Henriques' observations. There is no obvious reason why our first procedure (collection of the specimen at the maximum concentration) should not be applicable when the blood mixture has to traverse a capillary area, especially one with such a short and relatively constant circulation time as the lungs. To be sure, the period of increasing concentration before the maximum is reached will be somewhat lengthened, but it is difficult to see from the principle of the method how this can introduce an error so long as the steady maximum concentration lasts long enough for a good sample to be collected, which it certainly does with injection of sodium chloride solution.

Tigerstedt (14) appears to contend that because v. Kries (8) made experiments which confirmed the theoretical conclusion, that the mean velocity of a stream of water flowing through a straight capillary tube of certain dimensions is half the maximum velocity, owing to the uniform increase in velocity from the periphery to the axis, the circulation time through a capillary area, determined by noting the interval after injection of a salt at which the first portions of the salt arrive at a large vein draining the area, or in the case of the lungs at the carotid artery, must also be half of the mean circulation time. I am quite unable to understand how a given particle of salt solution, passing through a network of capillaries between corpuscles which are rubbing on the capillary walls, jostling each other and often temporarily blocking the capillary lumen, can remain always in the axial stream and always moving with the maximum velocity. It is much more probable that its velocity will vary considerably in its transit through the capillaries and will not differ much from the mean velocity of the whole of the blood. This conclusion finds confirmation in experiments performed on artificial capillaries (1).

TABLE 1

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*grams*	
1	16	10:48	71	115
		10:59	70	115
2	17.2	10:48	62	55
		10:57	58	55
		11:02	49	40
		11:13	45	25
		11:20	40	17
Average			51 ± 2.4; (σ 8)	
3	21	10:48	46	55
		10:54	42	30
		11:01	35	23
Average			41 ± 1.7	
4	8.5	11:05	113	85
		11:11	94	80
		11:19	78	40
		11:23	70	35
		11:27	57	35
		11:31	58	30
Average			78 ± 5.5; (σ 20)	
5	9	1:44	68	60
		1:48	86	70
		1:54	85	80
		1:58	82	80
		2:02	78	100
		2:06	81	100
		2:11	85	110
		2:16	84	105
		2:21	76	110
Average			80 ± 1.2; (σ 5.5)	
7	21		29	
8	22	10:34	40	
		10:39	39	
		11:28	48	
		11:33	35	
Average			41 ± 1.6; (σ 4.7)	
9	20		44	75*
			37	

* Blood pressure was raised by injection of strophanthin before the measurement.

My experiments on the output of the heart were planned fully as much to investigate the changes which might occur in its magnitude, when the circulatory conditions were greatly altered, as to obtain results which might be employed for calculating a so-called "normal average" for the output, a quantity whose variability is one of its chief characteristics. For this reason a number of the experiments were carried on to the point where the animal had markedly deteriorated and the circulation was failing. Experiments XVI and XIX (table 3) are samples. In the latter part of experiment XVI the pulse rate

TABLE 2

DOG	BODY WEIGHT	NUMBER OF OBSERVATIONS	DURATION OF EXPERIMENT	BLOOD DRAWN	SOLUTION INJECTED
	kgm.		*minutes*	*cc.*	*cc.*
XII	15.25	6	60	113	162
XIII	27.89	10	130	300	308
XIV	32.26	9	90	340	344
XV	4.97	4	110	53	71
XVI	11.79	10	95	250	251
XVII	18.20	12	125	220	207
XVIII	9.89	7	60	179	174
XIX	12.82	7	70	237	158
XX	10.32	5	50	148	92
XXI	14.99	9	140	184	193
XXII	17.5	16*	120	386	394
XXIII	7.15	5	40	91	81
XXIV†	34.55	11	145	300	245

In all the experiments the number of blood samples collected was approximately double the number of observations of the minute volume.

* Of these observations 5 had to be rejected because of unsatisfactory injection into the left ventricle, chiefly owing to insufficient pressure. In these 5 observations 27 cc. of the 1.5 per cent NaCl were introduced and 103 cc. of blood collected.

† Injection into descending aorta.

increased rapidly, the heart became progressively weaker and the output was soon cut down to one-third of its initial value. In experiment XIX the same thing was seen. The pulse became continually feebler, especially from the 4th observation to the 7th (inclusive). Precisely the same thing is seen in the experiments of Henriques (expers. 2, 3 and 4, table 1). The relative decrease in the output in his observations could not be quite as great, simply because the initial output, owing to the experimental conditions, was abnormally low, and the blood pressure rapidly fell to an extremely small value.

The opposite effect of artificially induced hydremic plethora is illustrated in experiment VII (table 3). In the course of the experiment 125 cc. of 5 per cent sodium chloride solution were injected through a catheter passed into the right external jugular vein. *In vitro* this would have trebled the molecular concentration of the original quantity of plasma. The conductivity of the blood drawn off at the end was

TABLE 3

EXPERIMENT XVI. WEIGHT 11.79 KGM.

Time	Minute volume per kilogram	Heart rate
	cc.	
1:57	318*	94
2:02	290*	99
2:09	233	96
2:17	174	120†
2:43	226	130
2:50	107	130
2:56	95	134
3:11	95	127
3:20	101	127
3:30	79	182
Average output (first 5 observations)		248
Average output (last 5 observations)		95

EXPERIMENT XIX. WEIGHT 12.82 KGM.

Time	Minute volume per kilogram	Heart rate
	cc.	
1:08	346	98
1:23	297	110
1:32	191	106
1:46	181	128
1:54	140	130
2:01	121	156
2:17	102	170

EXPERIMENT VII. WEIGHT 6.48 KGM.

Minute volume per kilogram.	Heart rate
cc.	
157	83
189	
226	98
295	97

* These two measurements are too high, as collection of the sample was begun simultaneously with the arrival of the first portion of the blood mixture before the sound had reached its maximum, and finished while it was still at the maximum.

† At this point the pulse rate increased abruptly and the heart beat became progressively weaker.

In experiment VII morphine and ether, in experiments XVI and XIX morphine and ACE mixture were the anesthetics.

twice as great as that of a sample taken at the beginning of the experiment. The progressive increase in the output is evident, the final measurement, while the animal was still in good condition, being nearly double the initial output.

The rest of my observations are summarized in table 4, which contains about 100 separate determinations on 14 dogs. There is reason

TABLE 4

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
XIV	32.26			68
			199[1]	84
			150	88
		4:24	146	92
		4:30	141	98
		4:45	170	98
		4:55	170	98
		5:05	159	146
		5:15	139	148
		5:25	169	150
Average			160 ± 4; (σ 11)	
Average (last 8 observations)			155 ± 3; (σ 12)	
XIII	27.89	3:30	110	68
		3:52	94	68
		4:09	146	70
		4:14	113	70
		4:26	115	70
		4:36	106	105[2]
		5:01	109	145
		5:11	79	158
		5:29	66	130
		5:40	54	136
Average (first 7 observations)			113 ± 4; (σ 19)	
XVII	18.20	3:30	147	66
		3:36	127	72
		3:49	118	53
		4:08	136	56
		4:16	165	56
		4:21	230[3]	58
		4:34	221[3]	59
		4:42	169	55
		5:07	129	72
		5:14	145	65
		5:25	134	59
		5:32	153	60
Average			153 ± 6; (σ 34)	
Average (omitting 6th and 7th observations)			139 ± 3; (σ 16)	

TABLE 4—*Continued*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE	
	kgm.		*cc.*		
XXII	17.5	11:52	221	69	
		12:03	270		
		12:11	221	74	Injection left
		12:20	175	72	heart
		12:52	265	72	
		1:07	166	72	
		1:11	235	72	
		1:26	203	69	
		1:31	214	73	Injection right
		1:39	175	80	heart
		1:45	185		
Average			212 ± 6; (σ 34)		
Average (first 7 observations)			222 ± 9; (σ 37)		
Average (last 4 observations)			194 ± 5; (σ 15)		
XII	15.25	3:40	199		
		3:49	192		
		3:57	156		
		4:11	191		
		4:20	218		
		4:35	207		
Average			194 ± 5; (σ 19)		
XXI	14.99	3:48	156	92	
		3:55	162	92	
		4:12	130	107	2% NaCl injected
		4:20	136	101	
		4:30	125	105	
		4:40	108	104	
		5:51	145	105	
		5:59	(187)[4]	104	1.5 %NaCl
		6:05	148	105	
Average			144 ± 5; (σ 22)		
Average (first 6 observations)			136 ± 5; (σ 20)		
Average (last 3 observations)			160 ± 7; (σ 19)		

TABLE 4—*Continued*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
X	12.29		211	
			184	58
			200	58
			(325)[5]	58
			205	64
			225	69
			205	69
			232	65
Average (omitting 4th observation)			209 ± 3.8; (σ 14.8)	
XI	11.68	3:30	214	72
		3:52	185	70
		4:51	(244)[6]	73
		5:17	207	89
		5:30	210	108
Average			212 ± 6; (σ 19)	
Average (omitting 3rd observation)			204 ± 4; (σ 11)	
XX	10.32	10:35	225	64
		10:46	251	74
		11:04	257	80
		11:12	193[7]	72
		11:21	232	77
Average			232 ± 7; (σ 22)	
XVIII	9.89	3:38	164	66
		3:44	137	62
		3:56	164	62
		4:03	148	64
		4:08	131	58
		4:26	136	74
		4:36	(116)[8]	78
Average			142 ± 4; (σ 16)	
Average (first 6 observations)			146 ± 3; (σ 13)	
IX	9.29		283	
			277	
			297	
			236	
			281	
			(181)[9]	119
Average			(259)	
Average (first 5 observations)			275 ± 6; (σ 20)	

TABLE 4—*Concluded*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
VIII	8.4		201	
			202	
XXIII	7.16	10:43	267	44
		10:51	235	37
		10:58	219	52
		11:15	269	49
		11:23	(163)[10]	48
Average			(211 ± 13)	
Average (first 4 observations)			247 ± 7; (σ 21)	
XV	4.97	3:00	317	82
		3:20	277	91
		4:45	331	74
		4:51	305	103
Average			307 ± 7; (σ 20)	

[1] Catheter somewhat obstructed by clot, so that only $\frac{1}{4}$ to $\frac{1}{5}$ of the usual quantity of salt solution ran into heart.

[2] Pulse rate abruptly increased; now unaffected by respiration (vagus paralysis). In the last observations pulse became weaker, and the circulation time from heart to femoral increased.

[3] Between 4:08 and 4:42 the output increased considerably and then diminished again (variations in anesthesia?).

[4] Pulse very strong. Time of injection only 8 seconds in this observation, as compared with 12 seconds in the others, and the quantity injected was probably too small for satisfactory measurement.

[5] This observation must be rejected owing to an error in resistance measurement of the sample. The data do not allow exact correction of the error, but the minute volume as given is certainly 50 per cent too high.

[6] The normal comparison sample for this observation was partially clotted, so that exact conductivity determinations could not be made.

[7] Collection finished too early, which would give too low an output as calculated by procedure II. The other observations were calculated on the rate at which the salt solution was injected (procedure I).

[8] The experiment was stopped by abnormally rapid clotting. The quantity of blood collected after injection of salt solution in this observation was too small for a fair sample.

[9] This observation should be omitted as it was made at the end, simply to test a technical point, a much smaller volume of a much stronger salt solution than was employed in the other observations of this experiment or in the other experiments being injected.

[10] The heart was failing at this point.

In experiments VIII to XIII and XV the animals were anesthetised by morphine and ether; in experiments XIV, XVII, XVIII, and XX to XXIII by morphine and ACE mixture.

to expect that the smaller animals should have a greater output per kilogram of bodyweight than the larger animals, in accordance with Rubner's results on the heat production in fasting dogs, which indicate a proportionality between the basal metabolism and surface area rather than between the basal metabolism and bodyweight. For this reason the experiments are arranged in the order of the bodyweights. Of course it would be desirable to have a much larger number of animals, especially at the two extremes, in order to test this point thoroughly.

It is well known that in experiments on different individuals of the same species made by the same method, the output per kilogram of bodyweight varies within wide limits. The variable response of different dogs to the experimental conditions, especially to the anesthetic, the differences in the build and breed of the animals and in the amount of fat entering into the bodyweight, as well as differences of age, size and other circumstances make it impossible to expect a close agreement in the average output of the different individuals in such a series as is given in the table. Even in non-anesthetised animals, as has been shown by Barcroft, Boycott, Dunn and Peters (9), and in man, as demonstrated by Krogh and his pupils (10), great variations occur. Muscular activity naturally exerts a great influence. The fact observed by Barcroft and his co-workers, that the proportion of the oxygen abstracted from the blood in the tissues may differ habitually in different individuals, must obscure any tendency to increase of output per unit of body weight in the smaller animals, until in a series of animals of progressively diminishing size the point is reached at which a physiological limit is set to any further abstraction of oxygen from, or accumulation of carbon dioxide in the blood. Above this point an animal with a habitually high coefficient of utilization may work with a lower cardiac minute volume than corresponds to its weight. But when the metabolism per unit of weight becomes several times as great in a small animal as in a large, it seems obvious that the minute volume of the heart must increase. Accordingly, a series of animals going down to quite small weights is most likely to reveal a relation between size and output per kilogram.

An important question is the degree of uniformity of successive determinations in the same animal, when no change is known to have occurred which is likely to affect the output. For the probable error of the average varies directly as the variability of the separate results and inversely as the square root of the number of observations dealt with. It will be observed that in regard to this criterion the averages deduced for

the separate experiments in table 4 must be considered satisfactory, although, of course, it would have been better to have a larger number of separate measurements from which to calculate the means. As Henriques justly remarks: "Eine Bedingung dafür dass die Methode benutzt werden kann, ist dass die injizierte Flüssigkeit tatsächlich mit dem Blut vermischt wird; dass dies mit Wahrscheinlichkeit stattfindet, hat Stewart in der obenerwähnten Abhandlung in hohem Grade wahrscheinlich gemacht, und die konstanten, mit der Methode gewonnenen Resultate, sprechen auch für eine solche Mischung."

It is all the more curious that Bock and Buchholtz (5), who speak with approval of the constancy of the results of Henriques (table 1), state that, "bei den schon erwähnten Untersuchungen von Stewart schwanken die bei demselben Tiere unmittelbar nacheinander ausgeführten Bestimmungen so weit voneinander, dass es sehr fraglich ist, ob man aus derartigen Einzelbestimmungen ein einigermassen richtiges Mittel erhalten kann." Their animals were anesthetised with a single dose of urethane and morphine at the beginning. The anesthesia was therefore constant during their short experiments. In my animals the anesthesia (morphine and then ether or ACE) was necessarily, and indeed purposely, more variable. Bock and Buchholtz cut the vagi, either at the beginning of the experiment or after one or two observations, and a good many of their animals were curarised. In my experiments the vagi were intact and no curare was given. The number of observations made by them on the same animal was generally much smaller than in our experiments. For all of these reasons it would not have been surprising if their individual observations did agree more closely. There is, however, little, if any real difference in this regard, as will be seen by comparison of table 4 with table 5, in which I have collected the measurements of Bock and Buchholtz and have calculated the averages with their probable errors and the standard deviation (σ). It will be noticed that these are precisely of the same order of magnitude as in my experiments (table 4). It should be explained that in many of the experiments of Bock and Buchholtz some change in the conditions was introduced between successive measurements (giving curare or section of the vagi, or administration of caffeine). But according to them none of these things affect the output, so that it did not seem worth while to swell the size of the table considerably by inserting them. The object of compiling it was to allow the reader at a glance to compare the results of the method in the hands of Bock and Buchholtz, under certain experimental condi-

TABLE 5

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*cc*	
1	22.5	4:02	140	180
		4:13	138	187
2	29		187	142
3	10.5		144	141
4	32.7	4:16	176	133
		4:27	179	147
		4:42	192	161
Average			182 ± 2.7	
5	32.5	3:20	228	160
		3:40	209	137
		4:19	206	97
		4:32	204	115
Average			212 ± 3; (σ 9)	
6	11.2	3:40	163	154
		4:30	173	129
		4:45	169	140
		5:08	137	109
		5:27	89	55
Average			146 ± 9; (σ 31)	
7	9.1	3:33	169	143
		3:45	164	159
		4:21	127	139
		4:57	137	163
		5:20	140	148
Average			147 ± 5; (σ 16)	
8	26.5	4:25	143	140
		4:38	173	167
		4:59	165	172
		5:25	175	173
		5:53	129	109
Average			157 ± 5; (σ 18)	
9	25	3:42	102	134
		4:18	117	134
Average			109 ± 3	

TABLE 5—*Concluded*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*cc.*	
10	25	3:50	234	151
		4:07	196	139
		4:19	228	137
		4:34	196	131
		5:02	188	117
Average			208 ± 6; (σ 19)	
11	17.5	3:42	159	140
		4:19	140	136
		4:36	127	125
		4:53	143	113
Average			142 ± 4; (σ 11)	
12	13	2:20	153	136
		2:30	161	128
		2:48	197	138
		3:12	161	135
Average			168 ± 6; (σ 17)	
13	19.8	3:54	114	164
		4:06	134	182
		4:25	179	176
		4:40	184	165
		5:20	194	120
Average			161 ± 9; (σ 31)	

tions, and in my hands under somewhat different conditions. It is clear that the results are substantially the same in the two sets of experiments. In the smallest dogs in table 4 there is a tendency for outputs somewhat higher than any in table 5 to appear. This is really a further rather striking confirmation of the general accuracy of the method since, as mentioned, we had a decidedly greater number of small animals than Bock and Buchholtz, and these small animals were generally not only small but not yet full grown. On the other hand, it would be idle to look in a series like this for a progressive increase in the output per kilogram as the body weight decreased. That would imply a uniformity of experimental conditions which is unattainable and a uniformity in the percentage of oxygen taken up by the blood

in passing through the lungs in the different individuals of the series which, as already mentioned, does not appear to exist (9). It is only feasible to compare the results, not only the averages but the extreme results, in fairly large groups of animals of very different size.

The averages deduced by Bock and Buchholtz by grouping their experiments in different ways also agree closely with mine, although they did not notice any special relation of output per kilogram to size. This could not be expected, as most of their 13 dogs were large and none really small. In 6 animals they found an average output of 157 cc. per kilogram per minute with intact vagi and without curare. Only one observation was made on each animal, except in one case where two were made. In the vagotomised dogs the average was 159 cc. per kilogram per minute. Comparison was not made in all cases in the same animal before and after section of the vagi. The variations in the output were from 179 to 114 cc. with intact vagi, and from 234 to 102 cc. after vagotomy. Their method of adding up all the separate observations and then taking the mean, instead of taking the mean of the average outputs for each dog, is objectionable, as it artificially weights any experiment in which more than one measurement happened to be made. No serious error, however, is introduced in the present instance, simply because only in one or two cases were even two observations made, the investigators usually contenting themselves with one measurement with intact vagi and one after vagotomy. As already mentioned, in a number of the animals no estimation of the output was made before the vagi were cut.

In their experiments on the curarised animals the output varied from 228 to 127 cc. per kilogram per minute; the mean, they say, lies "ein bischen" higher than in the non-curarised animals. As a matter of fact, if we average the 5 determinations in the way they did with the others, the average works out at 182 cc. per kilogram per minute. Even in the curarised animals considerable variations were found in estimations on the same animal, from 173 to 89 cc. per kilogram per minute in one experiment, the variation in this case being attributed by them to a change in the artificial respiration. In another animal, which was not curarised, but lightly anesthetised with morphine and urethane, the range was from 114 to 194 cc. per kilogram per minute. The chief part of the increase occurred after caffeine was administered, but the authors do not consider that caffeine has any definite effect upon the output, and attribute the marked variation in this case to the restlessness of the animal.

The highest output measured in their whole series was 234 cc. and the lowest 89 cc. per kilogram per minute. In all my observations on dogs weighing more than 10 kgm. (table 4), the greatest output was 270 cc. and the smallest 94 cc. per kilogram per minute, excluding one or two observations when the circulation was obviously failing. The greatest average output for these dogs was 232 cc., and the smallest 113 cc. per kilogram per minute.

This was also the smallest average output in the whole series. The animal (XIII) was a very fat spayed bitch weighing nearly 28 kgm. It ought really to be considered as a smaller animal carrying a great load of fat. This would artificially diminish the output per kilogram of normal bodyweight. Further, it is to be supposed that the metabolism was diminished by loss of the ovaries and the heat loss by the accumulation of fat. The organs (liver, spleen, kidneys, stomach and intestines) weighed, after bleeding, much less than the corresponding organs of other dogs of about the same weight. The heart also was probably somewhat small for the bodyweight. The thyroids were not enlarged. This is important to note in the case of dogs in the basin of the Great Lakes, as goiter is very common, accompanied by cardiac hypertrophy (goiter heart). This is best explained as a work hypertrophy associated with an increased cardiac output. The bloodflow through the thyroids per unit of mass is so great, and the circulation time from and back to the heart by this route so short that the additional work thrown on the heart by the presence of a large goiter might be as great as if an additional pair of hind limbs had been grafted on the neck. This is pointed out because in a series of dogs in this region the average output might perhaps be materially greater than in a corresponding series in a non-goiter region, even when animals with obviously large thyroids were rejected. While in pregnancy an increase of vascular territory is associated with an increase in blood volume (Spiegelberg and Gscheidlen, Rowntree et al.), it is not known, so far as I am aware, whether this is the case in simple goiter. The converse question, whether a great reduction in vascular territory in otherwise normal adults (after extensive amputations) is associated with reduction of blood volume, or whether the total average mass of the blood still retains its relation to the original dimensions of the body, has a certain general biological interest, and could probably be answered by means of the methods now employed for estimating blood volume in man.

The mean of the average outputs in the 6 dogs in my series weighing 15 kgm. or upwards was 156 cc. per kilogram per minute. Bock and Buchholtz, although one of their dogs weighed only 9.1 kgm., used mostly large animals, 9 out of 13 weighing from 17.5 to 32.5 kgm. The average weight of their 13 animals was 21.1 kgm.; the average weight of my 14 dogs, 14.2 kgm. The mean of the average outputs in my series of the 8 animals weighing 12.3 kgm. or less was 225 cc. per kilogram per minute. For the whole 14 dogs the mean of the average outputs was 195 cc. per kilogram per minute.

Treating the figures in another way, we get for 126 kgm. of animals (the 6 weighing 15 kgm. or more) a combined average output of 20.02 liters per minute, i.e., 159 cc. per kilogram per minute; for the 8 smaller dogs, weighing together 74.1 kgm., a combined average output of 15.97 liters per minute, i.e., 215 cc. per kilogram per minute. For the 14 dogs, weighing together 200 kgm., the combined average output was 36 liters per minute, or 180 cc. per kilogram per minute.

Bock and Buchholtz consider that the recent results of Barcroft and others (9) on unanesthetised goats, weighing from 14 to 38.6 kgm. (mean output 133 cc. per kilogram per minute for 21 animals; extreme range of the means 220 to 55 cc.) agree quite well (recht gut) with their own results on anesthetised, curarised and vagotomised dogs of about the same weight. The average weight of Barcroft's goats was 23.67 kgm., considerably more than that of the dogs of Bock and Buchholtz, and much more than the average of my dogs. One goat weighed 14.1 kgm., 4 from 17.3 to 19.5 kgm.; all the others were over 20 kgm. The largest single measurement of the minute volume was 220 cc.; the smallest, 46 cc. per kilogram.

Gréhant and Quinquaud (11) determined in 6 dogs, weighing from 6 to 18 kgm., the carbon dioxide content of blood obtained simultaneously from the right heart and the carotid artery, and also the amount of carbon dioxide given off per minute from the lungs, from which the quantity of blood passing through the lungs is calculated. Theoretically the arterial sample should be taken slightly later than the venous sample (at an interval equal to the circulation time from right ventricle to carotid). They apparently made only one determination on each dog. They do not give the bodyweights, but on the assumption that the smallest flow per minute per animal belongs to the smallest dog and the largest blood flow to the largest dog, we get 84 cc. per kilogram per minute, and 145 cc. per kilogram per minute respectively, for these two animals. But from the way the results are reported in the very brief note, this can only be a guess. It is therefore difficult to see on what grounds Bock and Buchholtz state that the results of Gréhant and Quinquaud appear to agree quite well (recht gut) with their own, whereas the observations of Zuntz were made with such an inadequate method that they can scarcely now be considered reliable. Zuntz used more than one method and one of these (12), in which the quantity of liquid which must be injected into the aorta during stoppage of the heart by the vagus in order to maintain the blood pressure at the original level is taken as equal to the output, can certainly not be con-

sidered adequate. But this criticism does not apply to Zuntz's experiments, carried out on the same principle (Fick's principle) as those of Gréhant and Quinquaud, but with estimation of the oxygen instead of the carbon dioxide. He found an output of about 73 cc. per kilogram per minute in a resting horse weighing 360 kgm., and about 90 cc. per kilogram per minute in resting dogs weighing 25 kgm. The observations of Barcroft and his collaborators were made by a similar method.

SUMMARY AND DISCUSSION

The recent results of Bock and Buchholtz (5), obtained by my method of estimating the output of the heart (but with injection of sodium iodide) are shown to agree well with the results obtained by the same method, with injection of sodium chloride. The two procedures according to which the method may be applied are discussed.

The first procedure, involving the collection of a sample of the blood mixture after the maximum concentration of the injected salt has been reached, and while the concentration remains steady at the maximum, was employed by the writer in the greater number of his observations, and later by Bock and Buchholtz. The quantity of blood entering the heart and mingling there with the injected salt solution is determined from the formula $V = Q\frac{60}{mT}$, where V is the volume of blood coming to the heart in a minute, Q, the volume in cubic centimeters of salt solution injected in T seconds and m the volume in cubic centimeters of the salt solution which must be added to 1 cc. of the normal blood sample taken before injection of the salt solution, in order to make its conductivity the same as that of the sample collected during passage of the blood mixture.

The second procedure was used in some of my observations and later by Henriques (4) (with injection of sodium sulphocyanide, instead of sodium chloride). It involves the collection of a sample of the blood mixture for the whole time during which it is passing the point of collection. On the assumption that the cardiac output remains approximately constant during the short time of an observation, no error is involved in commencing the collection at the moment when the injection of the salt into the heart is begun, as was done by Henriques. The output is calculated from the formula $V = Q\frac{60}{mt}$, where V is the minute volume, Q, the volume of salt solution injected in cubic centi-

meters, m, the volume of the solution which must be added to 1 cc. of the normal sample to make its conductivity the same as that of the sample collected during passage of the blood mixture, and t, the time of collection of the blood.

Any error dependent upon faulty collection with procedure I is most likely to give too high a value for the minute volume, and the opposite with procedure II. For in procedure I it is the maximum concentration which is to be determined. We cannot collect so as to obtain a higher concentration, but may in error start collection too soon or continue it too long. In either case a lower average concentration than the maximum would be given by the specimen, and therefore a value higher than the correct one would be calculated for the minute volume. With procedure II, if collection is begun before the arrival of the blood mixture no error results, but if it is begun slightly later than the arrival of the mixture and stopped before it has completely passed, a higher average concentration is obtained than if the sample is collected during the entire time of passage. The calculated minute volume will then be too low. So far as the avoidance of these errors is concerned, there may be some advantage in collecting a number of small successive samples during passage of the blood mixture. These can then be examined at leisure.

The general agreement in the results of the method in the experiments of Bock and Buchholtz and in those of the writer, although different substances were employed for injection, seems to indicate that it can be considered satisfactory for the study, in acute experiments on anesthetised animals, of the changes which the minute volume undergoes under the action of various factors, for instance, under the influence of drugs. A relatively large number of observations can be made on the same animal. It is essential to distinguish accidental changes, due to variations in the anesthesia or to respiratory or other movements, or to general deterioration of the animal with alterations in the blood pressure, from genuine effects of the factor under investigation. This may sometimes be difficult or impossible, and then the experiment must be rejected. The principle of the method is essentially the same as that originally suggested by Fick and used by Zuntz (13), and recently by Barcroft and his co-workers (9) in their experiments on unanesthetised goats, but instead of oxygen being added to the blood in the normal process of respiration, sodium chloride or some other substance is artificially added. The method is, of course, inferior to that employed by Barcroft in that it cannot easily be used

without anesthesia. It is perhaps barely possible that a satisfactory maximum concentration could be estimated by injecting into a vein through a needle, as now practised without anesthetics, a substance (vital red, e.g., or a drug used for intravenous medication) and collecting successive small specimens of blood by puncture of an artery, a procedure now also used to some extent in man (or in suitable animals by heart puncture). But even if possible, it would be quite another question whether such a method could be considered seriously in comparison with the gasometric methods. Whether with the refined technique introduced by Haldane, Barcroft and others for estimating blood gases and the respiratory exchange, gasometric methods would also be better even for observations on anesthetised animals, could only be decided by experiment, and perhaps the degree of experience of the individual investigator might be a matter of some importance.* Henriques states that he employed the injection method because he found the methods of Krogh and Lindhard not useful for animals. Theoretically, there is one point of superiority in the injection method as compared with Zuntz's method, namely, that the quantity of the substance added to the blood per unit of time is accurately determined, even for a period of a few seconds, by a simple volumetric reading. On the other hand, the addition of liquid to the blood, except in so far as this may aid in compensating for the blood withdrawn, is a drawback from which Zuntz's method is free.

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* Since this was written experiments on cats under urethane have been published by one of Barcroft's pupils. With the uniform anesthesia the values obtained for the minute volume in successive observations were admirably concordant.

STUDIES ON THE SECRETION OF THE PARS PLYORICA GASTRI

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Received for publication April 29, 1921

During the course of a series of studies on gastric ulcer one of us (1) devised an operation by which the pyloric portion of the stomach was isolated as a pouch with or without nerve supply intact. On making an analysis of the secretion of this pouch, our results showed discrepancies when compared with the reports in the literature and textbooks concerning the character of this secretion. Therefore an attempt toward a complete study of the physiology and character of the secretion of the pyloric portion of the stomach has been made.

Method. The operation for the production of the pouch will not be described here as it has been described in detail elsewhere (1).

The secretion was collected in a glass beaker enclosed in a copper cup (shaped like a silk hat) which was held in place about the opening of the pouch by means of a bandage.

All observations have been made on healthy animals, both immediately after operation and at periods up to as much as 18 months following operation. The nutrition of the animals is not disturbed in any way provided liquid and macerated food is fed for 3 to 4 weeks following the operation. Bones should not be allowed at any time as they have caused obstruction, not only at the orifice of the gastroenterostomy but as far down the intestine as 5 feet, in several animals of our series of over fifty successfully operated.

We believe that the secretion as we collect it from the pouch with or without the nerve supply intact is the normal secretion of the pyloric mucous membrane just as the secretion that is collected from the Pavlov pouch is the normal secretion of the fundic portion of the stomach. The mucous membrane is normal in appearance, being of the same color as the mucous membrane of the Pavlov pouch. Histological studies of the mucous membrane that were made and that will be referred

to later in the paper prove that the mucosa of the pouch is histologically normal. Studies on the absoprtion of the mucosa of the pyloric pouch, which are to be reported in a later communication, show the rate of absorption to be within the normal variations of the rate of absorption of the mucosa of the pyloric antrum isolated in situ by means of ligatures so placed as not to interfere with the normal blood supply.

PHYSIOLOGY OF THE SECRETION

Character of the secretion. The secretion is mucoid, viscous, tenacious, transparent, odorless and slightly salty in taste. In appearance and consistency it is best compared with egg white. On standing from 3 to 4 hours, or on centrifuging, a thinner, yet viscous, portion separates and rises.

Amount of secretion. In amount our observations compare favorably with those of Heidenhain (2), who reports a secretion of from 2 to 3 cc. per hour.

We find that the secretion varies in amount from 1 to 5 cc. per hour. The average secretion per hour for ten dogs with the nerve supply of the pouch severed is 2.25 cc., and for five dogs with the nerve supply of the pouch intact is 3 cc. We attach no significance to this slightly larger amount in the case of the pouches with the nerve supply intact. The secretion is more or less uniform in amount, but varies in different dogs and in the same dog from day to day.

In two animals which have been kept for 18 months following the operation the amount of secretion per hour in one of them is the same as it was a few weeks after the operation, in the other the amount of secretion per hour is about 75 per cent of the original amount. However, in the mucosa of the pouch of the second animal four experimental ulcers have been produced with resulting scar formation which might account for the diminution in the amount of the secretion.

Influence of meals and water drinking on the quantity of the secretion. Meals and water drinking do not influence the amount of the secretion, which is true for pouches with or without the nerve supply intact.

Heidenhain (2) reports that the secretion is slightly increased following the meals and reaches its maximum about the 5th hour. We were unable to confirm this report in a long series of experiments as any increase that occurred came within the normal variations of the rate of secretion and there was no constancy in the changes in the rate of secretion following a meal.

Diet does not influence the quantity of the secretion. Deprivation of water diminishes the amount of secretion, but the effect is not noticeable until after 24 hours.

Influence of "gastrin" and secretin on the quantity of the secretion. Proven active preparations of gastrin and secretin when injected subcutaneously do not increase the amount of the secretion or alter it in any way.

When gastrin is fed to the animals no increase in secretion results. (One hundred cubic centimeters were fed.) When gastrin is applied to the mucosa of the pouch the rate of secretion is not altered.

Influence of acid and of "fundic" secretion on the quantity of the secretion. The application of N/10 HCl or of gastric secretion from a Pvalov pouch to the mucosa of the pouch for a period of 5 minutes augments the hourly secretion from two to three times. On the application of either of these substances to the mucosa of the pouch hyperemia or congestion of the mucosa occurs, which probably accounts for the increase in the amount of the secretion. If the mucous membrane is gently massaged for 15 to 20 minutes with a cotton sponge soaked with N/10 HCl, it begins to bleed, the blood apparently oozing out instead of coming from some visible excoriated area. If gastric secretion from a Pavlov pouch is used instead of acid, bleeding does not occur as readily even though the titratable acidity is as great as that of the N/10 HCl. This bleeding only occurs on the outer or exposed edge of the pouch where the mucosa is subjected to more trauma (no visible lesions being present) and to the removal of the mucus secreted. The acid or gastric juice does not cause bleeding when applied for a long time to the unexposed portion of the pouch even though the same amount of massage is used.

Reaction of the secretion. The secretion reacts slightly aklaline, giving a pH varying from 7.00 to 7.50 when measured by the gas chain and colorimetric methods. It is neutral to litmus in most instances.

Heidenhain (2), Klemensiewicz (3) and Akerman (4) reported the secretion to be alkaline. Contejean (5) reported the secretion to be acid and explained the results of the first two investigators as due to atrophy of the mucosa of the pouch. This explanation is hardly tenable for we (6) have made histological study of the mucous membrane of the pouch and find no change even after 10 months. Contejean's findings of an acid secretion can be easily explained by a possible inclusion of some fundic mucous membrane in the pouch he made.

Is pepsin present in the secretion of the pyloric mucous membrane? Edkins (7) states that "it may be regarded as established that the pyloric glands do secrete pepsin." His statement is based on the physiological observations of Heidenhain (2), Klemensiewicz (3), Akerman (4), Contejean (5), Bergman (8), Ebstein and Grutzner (9) and Langley (10) and on the histological observations of Nussbaum (11), Langley (12), Stohr (13) and Oppel (14). Klug (15), Glaessner (16), Wassmann (17) and Wittich (18) report that any pepsin that was present in the pyloric mucous membrane was due to infiltration or absorption. All of this early work is controversial and is open to criticism in light of modern methods. Later histological investigation by Bensley (20) and Harvey (21) shows that neither parietal nor ferment cells are present in the mucosa of the pyloric portion of the stomach and that the line of demarcation between fundic mucosa and pyloric mucosa is rather sharply defined. Bensley (19) states that "the theory of Heidenhain, that the cells of the pyloric glands are pepsin-forming elements similar in character to the chief cells of the body of the fundus gland, has been shown to be incorrect" and that histological evidence indicates "that the bulk of the secretion of the pyloric gland is simply mucus." In our histological study of the pyloric mucous membrane we have failed to find either parietal or chief cells.

In a preliminary report by one of us (22) it was stated that a small amount of pepsin was present in the secretion. In the first five dogs operated we obtained peptic digestion of from 0.5 to 1 mm., using Schiff's modification of Mett's method. We also noticed a slight increase in peptic activity of the secretion in the 3rd and 4th hours after a meal and by the subcutaneous injection of gastrin. Since making the preliminary report the secretion of twelve other dogs has been examined for pepsin with negative results. We believe that the presence of pepsin in the secretion of the first five dogs operated was due to the inclusion of some fundic mucosa in the pouch due to insufficient care in the separating the pyloric portion from the fundic portion of the stomach. We believe that the presence of pepsin in the secretion of the two or three dogs reported by Heidenhain (2) can be explained in the same way. Akerman's technique for making the pyloric pouch can hardly be criticised. He only had one dog to live, however, and his method for determining the presence of pepsin is open to criticism in the light of modern methods and knowledge.

We also fail to find pepsin in the secretion from a pouch whose nerve supply is intact, which answers a possible criticism that the absence of

pepsin is due to the severing of the nerve supply, which objection has been raised by Matthews (23) against Heidenhain's observations.

The secretion has been examined immediately after the operation and at intervals up to as long as 18 months after the operation, pepsin being found at no time.

Is pepsinogen present in the pyloric mucous membrane? The mucous membrane of the pouch was treated with N/10 HCl and also with inactivated gastric juice for 5 minutes to ascertain if the contact of the HCl or inactivated gastric juice might not activate a possible pro-enzyme in the cells of the pyloric mucosa. As a result of this procedure the secretion was increased in amount from two to three times and rendered less viscous, but no peptic activity was present.

Also, as pointed out before, when the pyloric secretion itself is treated with N/10 HCl for activation of a possible pepsinogen, no digestion resulted in Mett's tubes.

TABLE 1

Showing effect of pyloric secretion on peptic activity

	SERIES A DIGESTION IN MILLIMETERS	SERIES B DIGESTION IN MILLIMETERS
First day	1.52	1.57
Second day	1.72	1.70
Third day	1.52	1.53
Fourth day	1.82	1.70
Fifth day	1.52	1.56
Average for 5 days	1.62	1.61

Effect of pyloric secretion on the peptic activity of fundic secretion. To ascertain the effect of pyloric secretion on the peptic activity of the fundic secretion two series of tubes were set up. One, A, containing 1 cc. of fundic secretion (obatined from a Pavlov pouch), 1 cc. of pyloric secretion and 15 cc. of N/10 HCl; the other, B, containing 1 cc. of fundic secretion, 1 cc. of distilled water and 15 cc. of N/10 HCl. To each series Mett's tubes were added. Both series were incubated at 37.5° C. for 24 hours. The pyloric secretion of two dogs on five successive days was used. The results are shown in table 1 and demonstrate that the pyloric secretion has no effect on the peptic activity of the fundic secretion. In other words, *the pyloric secretion does not contain an anti-peptic ferment.*

Is there a proteolytic enzyme present in the secretion other than pepsin?

Even though the secretion does not contain pepsin in any form, it is possible that it might contain a proteolytic enzyme similar to erepsin or trypsin acting in an alkaline medium.

By using Sorensen's method for erepsin and trypsin with large quantities of pyloric secretion (10 cc.) we were unable to find any evidence of the presence of either erepsin or trypsin in the secretion. The tests were carried out under absolute aseptic procedure, using toluol-treated secretion. If the secretion was not treated with toluol, digestion always occurred, as would be expected as the secretion is teeming with bacteria.

Is diastase present in the secretion? Klemensiewicz (3) reported that the secretion contained diastase. Heidenhain (2) failed to find it.

In our examination for diastase the secretion was treated with toluol and sterilized apparatus was used to prevent bacterial action. To 4 cc. of sterile starch paste solution 1 cc. of toluol-treated secretion was added. The reaction of the mixture was slightly alkaline (pH 7.5) in one series, neutral in another and distinctly acid to litmus (N/40) in another. The mixture was then kept at 38°C. for ½ hour and incubated for 3 hours at 38°C. At the end of this time a trace of dextrin was found when compared with the control.

We doubted that this trace of diastase was a product of the cells of the mucous membrane; but rather thought that it was only a filtrate from the blood. To determine this we injected intravenously Taka-diastase and found the diastase content of the secretion to vary directly with the amount of diastase injected. Hence we are of the opinion that the trace of diastase in the pyloric secretion is from the blood and is not formed by the cells of the pyloric mucous membrane.

Is invertase present in the secretion? Klemensiewicz (3) reported that invertase was present in the secretion. His secretion was mixed with pus, so his results are to be questioned.

All of our observations, made under antibacterial and sterile procedure, failed to demonstrate the presence of an invertase. One series of tests was made with the reaction of the mixture of saccharose solution and secretion slightly alkaline (pH 7.3) and another series with the reaction distinctly acid to litmus (N/40 HCl).

Is maltase present in the secretion? All of our tests for the presence of maltase in the secretion were negative. The same precaution of asepsis was taken and the importance of the reaction of the mixture was considered as was done in the case of diastase and invertase.

Is lipase present in the secretion? We were unable to detect any lipase in the secretion when antibacterial procedure was followed. The litmus milk and litmus olive oil tests were used.

Is rennin present in the secretion? Heidenhain (2), Klemensiewicz (3), Akerman (4) and Contejean (5) reported that rennin is present in the secretion of the pyloric mucous membrane.

In all of our observations we were unable to demonstrate the presence of rennin when 1 cc .of the secretion was incubated at 37° C. with 5 cc. of milk for periods of from $\frac{1}{2}$ to 3 hours. The secretion in another series of tests was activated by incubating the secretion with N/10 HCl for periods varying from 15 minutes to 1 hour and then neutralizing it with sodium bicarbonate solution. This activated secretion failed to cause coagulation. As much as 5 and 10 cc. of the secretion were used with the same negative results.

The influence of pyloric secretion on the coagulation of milk by rennin was studied. It was found that the secretion retarded slightly the coagulation of milk by rennin, but not any more, however, than the same amount of distilled water. The clot formed in the presence of pyloric secretion is less compact than the clot formed in the absence of the secretion. This was also found to be true in the coagulation of milk by gastric juice from a Pavlov pouch, i.e., if pyloric juice was added to the gastric juice, the resulting clot was less compact than with gastric juice alone.

It is to be recalled that Heidenhain, Akerman and the above mentioned investigators found pepsin in the secretion of their pouches, hence rennin would also be expected. We explain the presence of rennin and pepsin in the secretion of the pouches of the above investigators as due to the inclusion of some fundic mucous membrane in the pouches they made. Contejean found acid along with the pepsin and rennin. Heidenhain, Ackerman and Klemensiewicz found pepsin and rennin but no acid. Our explanation as to why they did not get acid associated with the pepsin and rennin is that physiologically their animals may have been in such a condition of health that no acid was formed. We met this possible objection by making five two-gastric-pouch-dogs, consisting of a pouch of the pyloric antrum and a Pavlov pouch, and collecting the secretion of the two pouches simultaneously. The Pavlov pouch gave the characteristic highly acid-pepsin-rennin secretion while the pyloric pouch gave the characteristic secretion described in this paper.

Anaphylaxis and precipitin formation? Two rabbits were injected intravenously on alternate days with 5 cc. of the secretion for a period of 2 weeks, following which the animals' serum was examined for a precipitin of pyloric juice, with negative results. An attempt was also made to sensitize two guinea pigs and a rabbit against the juice, with

negative results. The technique used by Elliot (25) was followed for both precipitins and anaphylaxis.

The speculative purpose of these experiments was to determine if the juice contained a protein that would cause antibody formation. Wells (24) states that any enzymatic secretion injected results in the formation of antibodies. Elliot (25) reports that mucin is an anaphylactogen. He prepared the gastric mucin he used by extracting the mucosa of the pig's stomach, while we worked solely with the secretion of the mucosa. Carlson (26) was not able, however, to sensitize an animal against human gastric juice.

Autodigestion? If the secretion is allowed to stand in an incubator for several hours, it becomes less viscous and reacts more acid. Such a change suggests a possible autodigestion as reported by Glaessner (16). But when toluol-treated secretion is incubated for 4 hours the above changes do not occur and there is no change in the color of the biuret reaction, or in the amount of alcohol precipitable mucin or proteins. So the liquefaction and change in reaction of the secretion on standing are most probably due to bacterial action, as microscopically many bacteria are present in the secretion.

Is secretagogue present in the pyloric secretion? Ten cubic centimeters of the fresh pyloric secretion have been injected a number of times both intravenously and deep intramuscularly into a Pavlov pouch dog without stimulating or having any effect on the secretion of the Pavlov pouch. Also 100 cc. of the clear pyloric secretion were incubated with 100 cc. of N/10 HCl and extracted as in the preparation of gastrin. Ten cubic centimeters of this extract when injected intramuscularly failed to cause any effect on the gastric secretion of a Pavlov pouch dog. Further, 100 cc. of the clear pyloric secretion was incubated with 100 cc. of gastric juice collected from a Pavlov pouch and extracted as in the preparation of gastrin. This extract also failed to cause any effect when injected. One hundred cubic centimeters of pyloric secretion were given to a Pavlov pouch dog by mouth without any stimulation of gastric secretion resulting.

This evidence is interpreted as showing that the secretion does not contain a secretagogue. Koch, Luckhardt and Keeton (27) showed this to be true for fundic secretion from a Pavlov pouch which makes the conclusion possible that none of the external secretions of the gastric mucous membrane contain secretagogues.

CHEMISTRY OF THE PYLORIC SECRETION

The results of chemical analyses up to date are shown in table 2. All analyses have been made upon clear secretion free from cellular detritus. The results reported (table 2) are the maximum and minimum found for the secretion of six different dogs. The analyses are expressed in grams per 100 cc. of secretion.

The specific gravity of the secretion as observed by us compares with that reported for the secretion by Klemensiewicz (3) (1.009 to 1.010). The osmotic pressure is slightly greater than that of the blood and "fundic" gastric juice of the dog as reported by Carlson (25). In this connection attention should be called to the chloride content of the secretion, which is also slightly higher than that of blood and "fundic" gastric juice, the average of our figures being 0.632 gm. per 100 cc. of secretion. It is interesting to note that the amino-acid nitrogen is the most constant of the substances of the analyses.

TABLE 2

*Showing the results of the chemical analyses of pyloric secretion grams, per 100 cc. of secretion**

	MAXIMUM	MINIMUM
Total solids	2.420	1.331
Total ash	1.540	0.558
Total nitrogen	0.095	0.054
Alcohol precipitable substances	1.120	0.896
Total chlorides	0.640	0.458
Total NH_3-nitrogen	0.0054	0.0033
Total amino-acid nitrogen	0.0025	0.0023
Specific gravity	1.011	1.008
Degrees of depression of freezing point	−0.640	−0.610

The biuret reaction gives a dark purple color.

* Only centrifuged samples were used.

SUMMARY

The secretion of the mucous membrane of the pyloric antrum is mucoid, viscous, tenacious, transparent, odorless and slightly salty in taste. The rate of formation varies from 1.0 to 5.0 cc. per hour and is not increased by meals, water drinking or secretagogues. Acids and irritants when applied to the mucous membrane excite the formation of the secretion. The secretion is alkaline (pH 7.00 to 7.50) which confirms

the reports of Heidenhain, Klemensiewicz and Akerman. The secretion does not contain enzymes, which is contrary to the reports of Heidenhain, Klemensiewicz, Akerman and others. The findings of enzyme by the early investigators is explained by faulty operative technique, the presence of pus in their secretion, bacterial enzymatic action and inadequate methods for determining enzymatic activity. The secretion does not contain an antipeptic ferment and does not form antibodies when injected parenterally. A gastric secretagogue is not present in the secretion. The results of chemical analyses are reported.

Hence our studies on the physiology of the pyloric secretion up to the present time substantiate the conclusion reached by Bensley (19) from a histological study of the cells of the mucosa of the pyloric antrum that "the secretion of the pyloric gland is simply mucus"; and we further add that it contains no substance that is of specific digestive importance.

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STUDIES IN NUTRITION

IX. The Nutritive Value of the Proteins from the Chinese and Georgia Velvet Beans

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Received for publication May 4, 1921

Chemical studies of the proteins of the Chinese and Georgia velvet beans have already been reported from this laboratory (1), (2), (3). The results obtained indicated that the amino-acid content of the proteins from these seeds was biologically adequate. When the cooked or autoclaved bean meal, however, furnished the only source of protein in an otherwise complete diet and was fed to albino rats, there was little or no growth. The addition of cystine or casein did not improve the diet in this respect. A ration prepared from the protein obtained by dialysis[1] was also inadequate for normal growth. The animals on these diets all showed signs of malnutrition. Their fur was ragged and their diets apparently caused regurgitation and diarrhea. That it is a question of digestibility or toxicity is indicated by our nutrition experiments with the protein obtained by heat coagulation[2] from either the Chinese

[1] The dialyzed protein was prepared by dialyzing in running tap water the clear 10 per cent sodium chloride extract of the bean meal. The protein which precipitated was washed with distilled water until free from chlorides. The protein was dried by treating successively with absolute alcohol and absolute ether. The ether was removed by heating the protein in a vacuum oven for a few hours at 110°C.

[2] The coagulated protein obtained by heating a clear 10 per cent sodium chloride extract of the meal at 105°C. until no more protein coagulated, was collected on a folded filter. The coagulum was suspended in distilled water and washed by decantation until free from chlorides. It was dehydrated by treatment with absolute alcohol and finally with absolute ether. The latter was removed by drying for a few hours at 110°C.

Both the dialyzed and the coagulated proteins are mixtures of two globulins differing chiefly in their sulphur and nitrogen content as well as their solubility in ammonium sulphate solutions.

or Georgia velvet bean. The protein prepared in this manner and supplemented with the other necessary dietary ingredients produced normal growth when fed to albino rats.

Miller (4) has isolated free dihydroxyphenylalanine from the seeds of the Georgia velvet bean. This amino acid has been reported to cause vomiting, and its presence probably accounts for the nutritional disturbances found in our experiments with the cooked or autoclaved bean meal. This would not account, however, for the failure of the rats to grow on the dialyzed velvet bean proteins.

It has previously been shown, in the case of the navy bean, that its proteins require cooking (5) in order that they may become available for the normal growth of albino rats, and that the improvement thus secured is probably due to an increase in digestibility (6). Preliminary experiments, made by H. C. Waterman of this Laboratory in a study of the digestibility *in vitro* of the velvet bean proteins, make this explanation the most probable one. The protein prepared by dialysis from the Chinese velvet bean was digested but little more than one-half as much as was that made by coagulation from the same bean. The figure obtained from the coagulum agrees well with those given by *cooked* phaseolin (6) and by casein. The good growth secured with the coagulated protein could then be explained as due to an increased digestibility, resulting from the boiling, in faintly acid solution, which was necessary to effect coagulation. It should be borne in mind, however, that a protein toxicity or an adsorbed toxic substance might also account for the failure of the dialysis product; and that the one might have been destroyed or the other removed in the preparation of the coagulum.

Scott (7), (8) has reported that ground velvet bean feed, containing both the seeds and pods, could replace an equivalent amount of peanut meal in a ration fed to cattle with no apparent ill effects; when fed to pigs results were obtained in some cases which indicated that it may cause abortion. Sure and Read (9), in a preliminary announcement of their experiments, suggest that the Georgia velvet bean is toxic in high concentration, impairing both growth and reproduction. They also state that the proteins from this seed were biologically deficient.

Experiments with cooked and autoclaved Georgia velvet bean meal. A diet prepared from cooked[3] Georgia velvet bean meal supplemented

[3] A thin paste was made by adding distilled water to the finely-ground beans. This was then cooked for 2 hours in a double boiler. The cooked meal was dried at a temperature of 60°C. and again ground to a fine powder, which was incorporated in the experimental diets.

by the addition of cystine,[4] Osborne and Mendel's salt mixture, butter fat and lard, was fed to albino rats. After a short time on this diet the animals became sickly in appearance, their fur was ragged and but very little growth occurred. The regurgitated velvet bean meal produced dark stains under their bodies. This may have been due to the oxidation of free dihydroxyphenylalanine in the regurgitated velvet bean meal; Miller (4) found that the aqueous extract of the bean meal darkened on exposure to the air, and it was shown that this darkening was

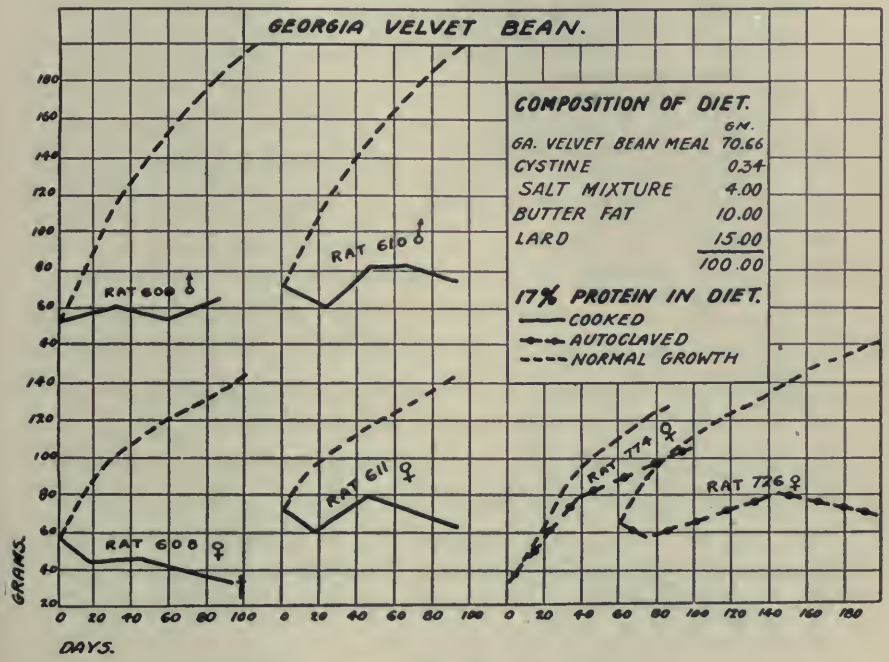

Chart 1

due to the oxidation of dihydroxyphenylalanine. There was a slight improvement in appearance of the animals when fed a diet similar except that the bean meal was autoclaved[5] at 15 pounds for one hour. The composition of these diets and growth curves are shown in chart 1.

[4] The cystine used in these experiments was prepared by Mr. S. Phillips of the Protein Investigation Laboratory, Bureau of Chemistry.

[5] The autoclaved bean meal was dried at 60°C. after removing from the pressure cooker, and reground to a fine meal.

Experiments with cooked and autoclaved Georgia velvet bean meal supplemented with casein. In order to eliminate the possibility of deficient proteins, casein was incorporated in the diet of cooked velvet bean meal, to which the other necessary dietary ingredients were added. No appar-

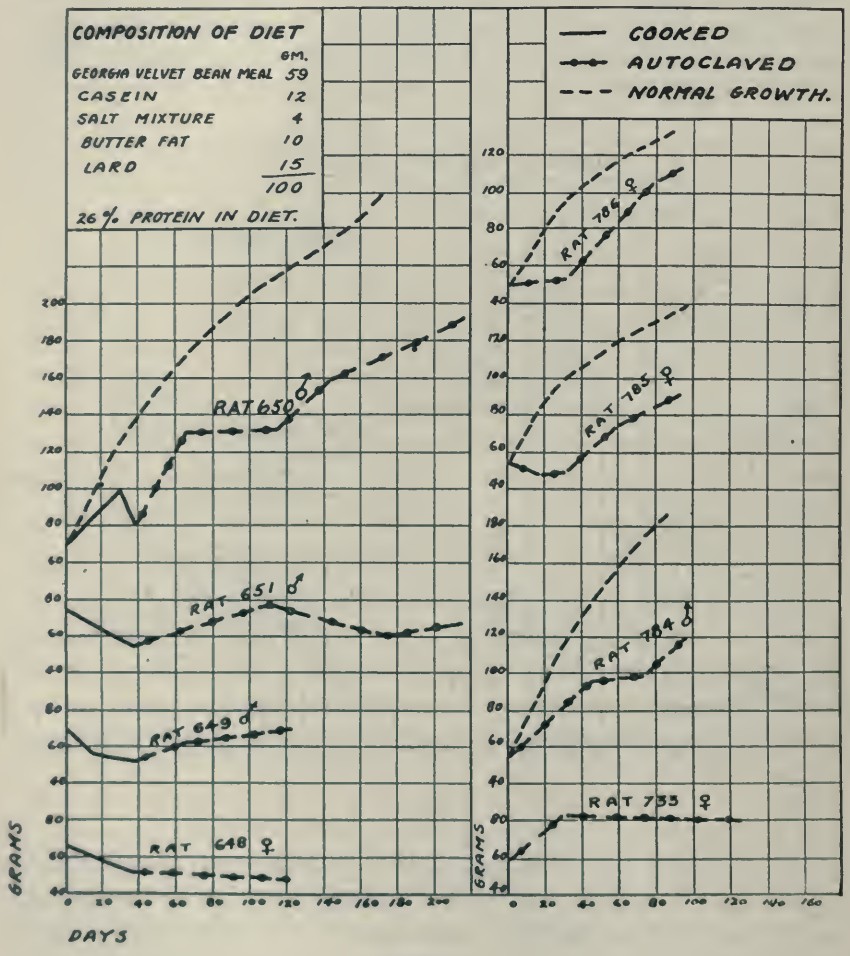

Chart 2

ent change was noted between the group fed the diet containing casein and those not receiving the additional protein. With a similar diet in which autoclaved meal was fed, it was noted that while the rats did not grow at the normal rate, regurgitating did not occur. The appear-

ance of the animals in this group was also noticeably better. The results of these experiments indicate that some toxic substance was responsible for the regurgitation and, in part at least, for the lack of growth. Heating apparently improved the diet with respect to the former disturbing factor. The results of these experiments are shown on chart 2.

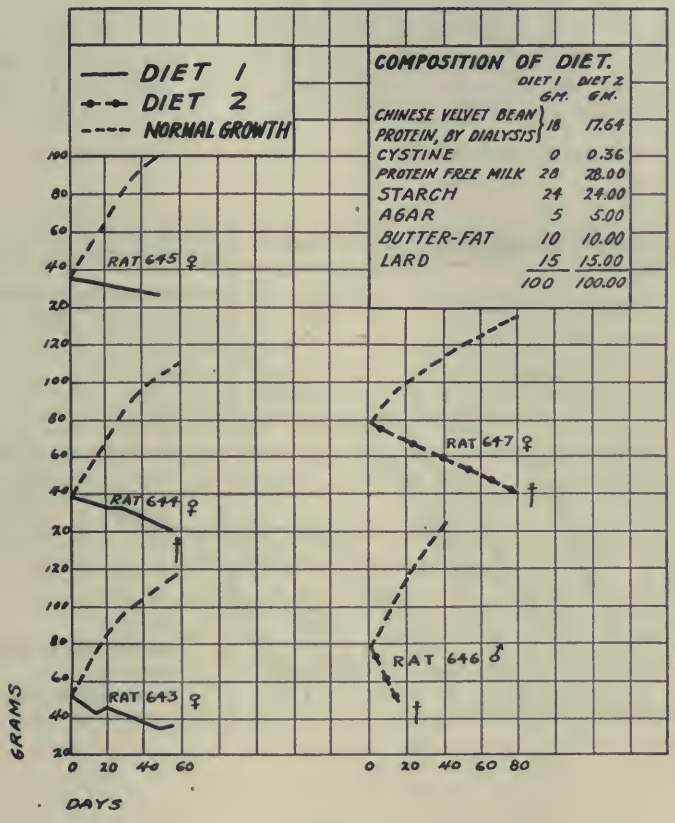

Chart 3

Growth experiments with the isolated protein from the Chinese and Georgia velvet beans. A number of experiments were made with the isolated protein from the Chinese velvet bean which was prepared by dialysis. If a toxic substance present only in the seeds but not necessarily in a combination with the protein was the disturbing factor, then a diet prepared with isolated protein, to which was added the other necessary dietary ingredients, should eliminate the question of toxicity. The

animals placed on such a diet, however, lost rapidly in weight although their food intake was normal. Previous experience with the navy (5), adsuki (10) and lima (11) beans had shown us that the proteins of these seeds were deficient in cystine. Addition of cystine, however, did not improve the results obtained from the dialyzed velvet bean protein diet; the animals lost rapidly in weight and died.

Entirely different results were obtained, on the other hand, when the protein prepared by coagulation with heat was fed. Normal growth occurred when the source of proteins[6] was either from the Chinese or Georgia velvet beans. The addition of cystine was not required.

The growth curves and composition of the diets are recorded on charts 3 and 4.

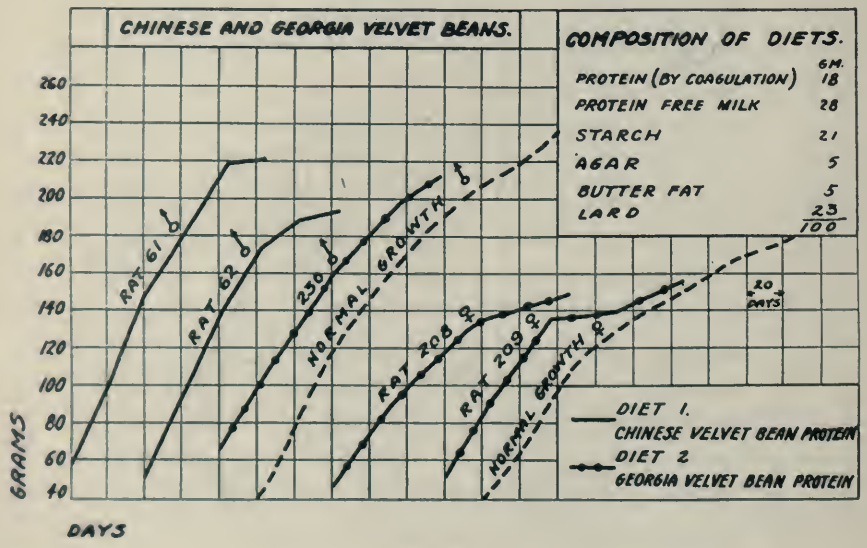

Chart 4

SUMMARY

1. The isolated proteins obtained by coagulation with heat from either the Chinese or Georgia velvet beans are adequate for the normal growth of albino rats. Chinese velvet bean protein prepared by dialysis, however, whether or not cystine was added, resulted in lack of

[6] The protein was isolated from a different sample of beans than that which was used in the feeding experiments. The beans used in this investigation were furnished by the Bureau of Plant Industry of the United States Department of Agriculture.

growth. The bean meal, on the other hand, cooked or autoclaved and supplemented with either cystine or casein, resulted in nutritional failure, manifesting itself in lack of growth, regurgitation, diarrhea and ultimately death.

2. The relative probability of protein toxicity, associated toxic substance (dihydroxyphenylalanine ?), or partial indigestibility of the protein, as causes of the failure of the dialyzed preparation, are discussed. An associated toxic substance seems the most probable source of the nutritional disturbances observed in the case of the steam-cooked meal.

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THE MECHANISM OF THE RECOVERY OR MAINTENANCE OF SYSTEMIC BLOOD PRESSURE AFTER COMPLETE TRANSECTION OF THE SPINAL CORD[1]

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Received for publication May 4, 1921

Physiologists have been divided in opinion for years as to the criteria to be observed in arriving at an estimate of the function of particular levels or regions of the central nervous system. The methods of experimentation most commonly employed involve anatomical destruction of some part, or anatomical solution of continuity of conduction pathways in the central system. Two stages may be observed in the course of the recovery of an animal from an experimental lesion of any portion of the nervous system; there is first the acute state, during which the effects of the lesion are most severe or most noticeable. Following this, there is a period in which the effects grow generally less severe or less noticeable until a state is reached at which no further change occurs. The effects observed in the first state, but which disappear in the course of the second or recovery period, are commonly attributed to shock. The progress during the second period is often attributed to the general failure of the shock effect. The ultimate state of recovery, in which there is the deficiency of function, is taken as the truest index of the function of the injured portion of the nervous system. But there is no general agreement even here, for some insist that if shock could be completely eliminated, the recovery would be still more complete, while others feel inclined to set a limit to the effects of shock, and consider that the minimal deficiencies of function indicate the complete subsi-

[1] The author gratefully acknowledges her indebtedness to Prof. W. T. Porter for the generous hospitality of his laboratory in which these experiments were done, and to Prof. F. H. Pike for his guidance and help throughout the work. The paper was submitted as a thesis for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

dence of all shock effects, and are therefore to be taken as the true criteria of the function of the injured portions of the nervous system.

There is in the literature no consistent statement of the functional organization of the nervous system. According to the importance one attributes to such vague and generally unmeasured influences as shock, depression, loss of tonus and other like terms for unknown processes, one may take either of two positions and ally oneself with either of two general hypotheses of functional organization. Briefly stated, these two positions are:

1. The segmental hypothesis of Goltz and his followers, in which shock plays an all important rôle. In its general form, the hypothesis states that the same level or division of the nervous system has essentially the same function in all types of animals. The reason that a dog does not recover as completely as a frog after decerebration is that shock is more severe and more persistent in the dog. No limit is assigned to the effects of shock. One important consequence of this hypothetical omnipotence of shock is that there is, according to this hypothesis, no localization of motor function in the cerebrum.

2. The theory or hypothesis of cerebral localization. The most extensive statement of this theory at the present time is due to von Monakow. He clearly recognizes that one must assign some limits to the effects of shock, and takes the position that all the effects of a lesion in the central nervous system of a mammal—for example, a dog—are not due to shock. He considers that the shock effect ultimately recedes completely, and that the final deficiencies of function observed after a considerable period has elapsed are to be taken as an index of the function of the lost or injured portions of the nervous system. An important conclusion is that there has been a shifting of function in the phylogenetic development of vertebrates, and that we have phylogenetically new, as well as phylogenetically old, sensory and motor pathways for somatic functions.

A modification of this second position consists in limiting still further the effects of shock, and of introducing the idea, due to a suggestion of Hughlings Jackson, that there may be a quantitative change in the amount of nervous energy passing over the remaining pathways, or through the remaining levels, of the nervous system after an injury to one or more of them. It is obvious that a quantitative change, tending to an increase in the functional capacity of the remaining levels or pathways, might tend to decrease the final deficiency of function, and so lead to too low an estimate of the actual work of the injured portion. A fuller

discussion of these views is given by Pike (1). It may be remarked in passing that the question of shock is bound up with the question of the validity of our methods of investigation of the nervous system, and the soundness of our views on its function.

The difficulty in arriving at an estimate of the actual importance of shock, or of the effect of a quantitative change in the function of the remaining pathways after an experimental lesion, has arisen from the fact that it has been difficult, or generally impossible, to get at any independent quantitative determination of either factor in the equation. Until this is done, the solution of a single equation of two or more unknown quantities must remain indeterminate.

It is desirable to find some physiological system whose functional continuity may not be completely interrupted by a total transverse lesion of the spinal cord, and then to determine, after an interval of recovery, the effect of temporary or permanent elimination of the parts lying above or below the level of transection. If, then in investigating the functional organization of the nervous system by noting the results of lesions, it were possible to study the relationships in some system in which the outflow from the central system is widely distributed, one should be able, by isolating different segments of the outflow, to derive some information as to the functional significance of these different levels of the central mechanism relative to the system under consideration.

The vascular system fulfils these conditions admirably. It is generally agreed that a vasomotor center lies in the medulla. Efferent vasomotor fibers leave the spinal cord with the first or second thoracic roots through to the thirteenth thoracic root, the heaviest outflow, according to Langley, occurring in the region of the sixth thoracic root. No vasomotor fibers leave the cord in the cervical region and few or none in the lumbar and sacral regions.

In view of the fortunate anatomical arrangement of the efferent portion of the vasomotor system, it seemed feasible to effect an actual separation of the higher centers from the spinal cells by complete transection of the spinal cord.

In the normal life of the individual the blood pressure is maintained at a practically constant level through the cardiac nerves and the vasomotor mechanism. It is common knowledge that the immediate result of complete transection of the spinal cord (in the upper thoracic region) is a severe fall of blood pressure and a cessation of skeletal reflexes involving muscles whose afferent and efferent nerves enter or leave the spinal cord below the level of transection. The blood pressure does not return

to its normal level and only a part of the somatic reflexes return during the course of an acute experiment. In general the higher the level of transection of the thoracic cord, the greater the fall of the blood pressure. The severity of the effect is independent of the method of blocking conduction in the cord (2). If, however, the animal be kept alive for a period of days, there is a more extensive recovery of the skeletal reflexes and the blood pressure is said to approach the level maintained before the injury. The general statements in the literature are, however, too vague to permit of drawing any sound conclusions. More exact control data have recently been gathered which serve as a basis of comparison for the results given in this paper.

To what, we may ask, is this recovery due? If we cling to the segmental theory, we might advance the hypothesis, as Goltz did, that the recovery of blood pressure is due to the subsidence of the effects of shock which had depressed the spinal cells of origin of efferent vasomotor fibers lying below the level of transection. If, however, we believe that, in the development of the higher animal forms there has been a greater tendency toward cerebral localization, we might look to the center in the medulla oblongata and the outflow still in functional connection with this bulbar center for an explanation of the return of blood pressure to or maintenance at the higher level.

After allowing a period for recovery, one should be able to determine: *a*, whether or not there is a return of blood pressure; *b*, if so, to what extent recovery has taken place; and *c*, whether the mechanism on which the recovery of blood pressure depends, involves the medulla oblongata and the efferent vasomotor fibers above the level of transection, or the spinal cells below the level of transection from which efferent vasomotor fibers take their origin.

These three points comprise the objective of a series of experiments described in this paper. It was believed that by measuring the blood pressure at varying intervals after transection of the cord, the presence or absence of recovery and its extent should be shown; by functional elimination of the medulla oblongata through occlusion of the head arteries, specific information should be gained regarding the rôle of the medulla in maintaining this pressure, since it is difficult to see how any operation on the medulla can affect the isolated segment of the spinal cord. Measurements of the blood pressure obtained after cerebral anemia, the spinal cord being anatomically intact, served as a control for the general level of spinal pressure. If the spinal cells were responsible for the recovery of the blood pressure then the functional elimi-

nation of the medulla ought not to affect the level of blood pressure seriously. If, on the other hand, the recovery of blood pressure had occurred through the center in the medulla and the spinal cells were normally dependent on the higher center for their functional activity, elimination of the medulla should result in a considerable fall of blood pressure.

Before presenting the results obtained by the experimental procedures outlined above, a brief consideration of the literature which bears most directly on these points is advisable.

Goltz (3) transected the spinal cord of dogs between the thoracic and lumbar segments. Other reflexes than those involving the vasomotor mechanism were the object of the investigation and the observations on the effect of the lesion on vasomotor responses appear to be incidental. No actual measurement of blood pressure is recorded. Goltz noticed that after transection the hind feet became warmer, due to vasodilatation, and that gradually the difference in temperature of the fore and hind feet disappeared, and later the hind feet were even colder than the forefeet. Comparing these results with those previously obtained on frogs, he concluded that the lumbar cord was the vasomotor center for the hind limbs. Goltz assumed that the temporary abolition of reflexes after section of the cord was due to the suppression or inhibition of the normal function of the cells of the lower level of the cord by shock. What recovery of function occurred was due to the passing of the effects of shock, and failure to recover completely was attributed to the persistence of shock.

Twenty odd years later Goltz (4) made a series of experiments on dogs in which he removed a large part of the spinal cord, in successive operations. The upper level of transection, he states, was in some cases as high as the fifth or sixth cervical vertebra; it was less dangerous, however, and a more complete recovery occurred if the first transection was made in the region of the third thoracic segment. These animals lived for months apparently in good health, from which Goltz drew the conclusion that it was erroneous to assume that the spinal cord of warm-blooded animals was necessary for the carrying on of the processes of nutrition, for the regulation of the size of the blood vessels and the maintenance of body heat.

One might reasonably ask, then, what has become of the vasomotor center which had previously resided in the lumbar cord? Sherrington (5) states that complete transection of the spinal cord of the dog through the eighth cervical segment is followed by a severe fall in the general

arterial blood pressure. After some weeks the blood pressure, with the animals in the horizontal position, will often be found practically normal. The arterial pressure in one dog, taken 300 days after transection, was 90 mm. Hg. The blood pressure found is maintained by the spinal cord, and not by peripheral mechanisms, as is shown by the fact that a profound fall occurs on destruction of the cord in the thoracic region. One should, therefore, exercise some care in comparing the results of Goltz' experiments on destruction of the spinal cord where the transection is made at the level of the second or third thoracic segment, and a similar destruction where the transection is made at the level of the eighth cervical segment. The experiments reported here are related more closely to Goltz's findings than to the findings of Sherrington.

Langley (6) further investigated the existence of spinal vasomotor centers with the use of strychnine. The cats were decerebrated and the spinal cord transected in the mid cervical region. By this procedure all question of the possible influence of higher centers on blood pressure was eliminated since the level of transection was above the outflow of vasomotor fibers. Any change in blood pressure then would necessarily be attributed to the activity of the cells of origin situated in the spinal cord. On the injection of a small amount of strychnine a prompt and extensive rise of blood pressure was obtained. The conclusion was drawn that the strychnine had stimulated spinal vasomotor centers. Langley confirms Pike's (7) observation that the intravenous injection of curare is followed by a further fall in the blood pressure of the spinal animal. He considers this fall to result from a decrease of muscle tone. The justification for considering these spinal cells of origin of efferent vasomotor fibers as vasomotor centers is not clear. It is not to be denied that these cells may be stimulated in different ways to give vascular responses, but the real question is, what is the source of these impulses in the natural life of the animal? Do they come from higher centers or do the spinal cells possess an actual autonomy? Electrical stimulation of the cord, as well as strychnine, causes marked vasoconstriction, but neither fact offers sufficient ground for assigning independent vasomotor centers to the spinal cord.

If, as Langley states, fibers coming in through the dorsal roots make connection with the vasomotor cells in the spinal cord, the visceral sensory and motor system has an arrangement similar to that obtaining in the somatic sensory and motor neurones of the spinal cord. Such a similarity might be expected on general grounds, but the question of morphological relationship still remains to be answered. Some investi-

gators believe that the somatic sensory fibers send collaterals directly to the ventral horn cells. Such a connection in the case of the sympathetic system must be effected between the afferent fibers and the preganglionic cells in the cord. If the post-ganglionic cells of the sympathetic system correspond to the ventral horn cells of the somatic motor system, the regions of arborization between the afferent fibers and the motor cells may not lie at the same place in the two arcs—somatic and visceral.

On the other hand, there is striking evidence in favor of the localization of the vasomotor center in the medulla. Ranson (8) sectioned only the apices of the dorsal gray columns at the level of the first thoracic roots. In this way certain afferent vasomotor fibers alone were interrupted, efferent channels for vasomotor or skeletal reflexes being unaffected. Thus all possibility of what Von Monakow calls the diaschisis effect was ruled out, since diaschisis is held to produce its effect over efferent pathways alone. Vasomotor reflexes were tested by stimulation of the brachial and sciatic nerves. As a result of such a lesion the usual rise of blood pressure obtained on stimulation of the sciatic nerve was abolished or markedly decreased whereas the rise obtained from stimulation of the brachial nerve persisted in undiminished intensity. The interpretation placed on these results was that the pressor pathway from the lower level had been interrupted before reaching the higher centers, whereas the central connections of the pressor fibers entering the cord with the brachial nerve above the lesion were intact. Thus Ranson accounts for the difference in response of the two nerves which, in an uninjured animal, produce equal effects on stimulation.

THE EXPERIMENTAL METHODS, MATERIAL AND RESULTS

The material used and the preliminary operation. Half-grown cats, preferably females, were selected for operation when available. As a matter of fact, adult cats of both sexes were used at times. Ether was the anesthetic employed. The fur was clipped over the field of operation with scissors. The field was then scrubbed thoroughly with a solution of mercury bichloride 1:1000. Towels which had been boiled and wrung out of bichloride covered the surrounding parts. Instruments were sterilized by boiling.

A longitudinal incision was made in the median dorsal line in the cervical or thoracic region, depending on the level at which the transection was to be made. The muscles were cut from their attachments to the spinous processes, held back by means of a retractor and the vertebrae

scraped free of muscle. Usually the spinous processes and arches of two vertebrae were removed with bone forceps. Care was taken not to extend the removal of the arch of the vertebra more than 2 or 3 mm. from the median line lest the hemorrhage be too severe. The wound was thoroughly washed out with hot sterile water and dried with sterile cotton. The following method of cutting the spinal cord was found to be most successful. - A small pair of fine curved forceps was cautiously placed under the cord, which was then gently lifted partially out of the canal and a quick transverse section made with scissors. This procedure involved a slight hemorrhage from the anterior spinal vessels, but the bleeding soon subsided. The wound was then flushed clean with hot sterile water and closed with sutures. Sterile catgut or cotton thread was used. The wound was covered with sterile gauze and cotton and then bandaged. In no case did suppuration or meningitis occur.

Twenty-eight rabbits and one cat were used in the earlier experiments, but in no case was a completely successful measurement of blood pressure obtained. (A measurement without any operation affecting the medulla oblongata was made on one rabbit.) Nineteen cats were used in the final experiments, and measurements of blood pressure, partially or wholly successful, were made on twelve of them. Three other preliminary observations made by Mrs. Winkin, to be published by her, gave results in entire accord with mine.

The post-operative care and condition of the animals. Upon this hung the success or failure of the experiment. Snuffles, anthrax and pneumonia brought several to an untimely end, but these maladies could be attributed to pre-operative infection and not to any untoward result of the transection.

The immediate result of the transection was a severe drop in body temperature, which the animal was unable to bring back to normal by its own heat-regulating mechanism. It was placed on a bed of hay in a warm pan. An electric pad supported by the sides of the pan covered the cat and supplied the necessary heat. In one case the current was inadvertently turned off from the pad on the first night after the operation. In the morning the cat was very cold and weak. Heat was at once supplied, with favorable results, but after the failure of the heat on the second night the animal died. After 24 to 48 hours, depending on the condition of the cat, it was removed to the recovery cage. After the first 48 hours the heat-regulating mechanism appeared to regain its function in large part.

The heat-regulating mechanism of the rabbits, however, showed little tendency to recover its former efficiency. A severe fall of body temperature, ranging from 4 to 10°F., followed immediately upon transection of the cord. Furthermore, it was necessary in many cases to resort to the almost constant use of the electric pad in order to keep the temperature of the animals even approximately normal. The fluctuations of temperature were less marked after hemisection than after complete transection. This is shown by a comparison of the temperature variations in rabbit 10 during the period November 15 to November 25 and November 26 to December 1 and by a further comparison of the variations in rabbit 14. The results are tabulated below:

NUMBER OF EXPERIMENT	LOWER AND UPPER LIMITS OF BODY TEMPERATURE	
	After hemisection	After transection
10	96.4°–101.8°	92.2°–103.2°
14		92.8°–107.2°

The greater recovery of the heat-regulating mechanism in the cats may have been due largely to the return of muscle tone and the increased muscular activity, to be discussed in a subsequent paragraph. The lack of recovery in the rabbits, on the other hand, may bear some relation to the fact that the rabbits were comparatively inactive.

The cats were fed once a day, the food consisting of meat, fish heads, liver, and bread and water in which meat had been boiled. Appetites were good on the whole and in some cases the animals were greedy.

The bladder was emptied once a day by manual pressure. Considerable pressure was required for the first 2 or 3 days, particularly in male cats, but after that the urine could be expressed fairly easily. After 16 days or more there was a certain amount of spontaneous voiding of the urine. Complete spontaneous evacuation was not usual. In one cat which was pregnant, partial recovery of the automaticity of the bladder was noticed on the 4th day. After the 8th day the recovery was practically complete. Defecation occurred normally in all the animals.

The cats were rather active, some of them very active indeed. After the 1st to 3rd day, the muscles innervated from the isolated part of the spinal cord regained their tone in part. The flexors of the hind legs could be reflexly stimulated by pinching the toes, and a crossed extension reflex was produced by tapping the knee. Also on holding a cat that had survived the transection 16 days by the forelegs so that the hind legs hung free, the progression reflex of the hind legs was obtained. Cat

18, in which the transection was at the level of the seventh cervical segment, was unable to support itself on its forelegs.

In cat 14, which lived for 32 days after the transection of the spinal cord, the impact of the hind legs against the side of the cage as the animal dragged itself about by its fore limbs was often sufficient to produce strong extension of both hind limbs, upon which there was superposed a tremor with a frequency of about four or five in the second. This rarely lasted for more than a second.

Only two of the cats were afflicted with sores. Goltz remarked that these sores, occurring on the part of the body below the level of transection, were difficult to avoid and extremely difficult to heal if they appeared. In one case the sore appeared after 10 or 12 days, and in the other after 28 days. Both were on the thigh. It is probable that the sores came as a result of rubbing against the wire netting of the cage. This netting was covered with a thick bed of hay, but both of these cats were extraordinarily active and the netting was uncovered in places as they crawled about the floor. Furthermore, as the recovery of the mechanism for emptying the bladder progressed, it became impossible to keep the animals dry, and this acted as a further and continuous source of irritation.

Three cats, 3, 16 and 20, in which the level of transection was high, evinced sudden and grave signs of collapse, which proved fatal in one case. First signs of difficulty were observed, in the case of cats 3 and 16, when they were placed on their backs for the purpose of expressing the urine. Their heads were held down with extreme gentleness. In spite of this they became greatly agitated and began pawing violently with the fore legs and struggled to free themselves. Close upon the heels of this unusual exertion followed slow and labored breathing and even convulsive movements, as the respiration became more infrequent and gasping. The thoracic wall was compressed rhythmically in an effort to restore normal respiration. The efforts were attended by success in the case of cat 16 but cat 3 continued to manifest such serious dyspnoea that it was necessary to resort to artificial respiration. At this time the pupils were widely dilated and the corneal reflex absent. Under the more effective measure recovery was brought about, but rather than risk another collpase preparations were made to record the blood pressure at once. The protocol of the experiment follows.

Cat 3. Blood Pressure Tracing. February 20, 1920. A little ether—Tracheotomy—Heart slow and respiration bad.

8:05 p.m. Left vagus cut: Mean blood pressure 50 mm.

8:08 p.m. Right vagus cut. Corneal reflex present: No change. Momentary fall.
8:14 p.m. Ligation of cerebral arteries: Rise of 7 or 8 mm., reaching 55 mm.
8:18 p.m. Release of cerebral arteries: Pressure had fallen to 40 mm.
8:25 p.m. Abdominal aorta compressed: Rise from 40 to 130 mm. (N.B. good condition of heart.)
8:40 p.m. Stimulation of central end left vagus: no effect.
8:43 p.m. Stimulation of left sciatic: Fall of 5 mm., then slight rise to 50 mm. After stimulation stopped pressure fell to 44 mm.
8:45 p.m. Stimulation of central end left vagus: No effect.
8:47 p.m. Ligation of cerebral arteries (this ligation was apparently incomplete as respiration was not interrupted): Rise from 44 to 58 mm. returning to 44 mm. as final result of occlusion.
8:55 p.m. Readjustment of ligatures making ligation more complete: Rise from 40 to 48 mm.
9:00 p.m. Stimulation of sciatic: Rise from 44 to 50 mm.
9:05 p.m. Pressure gradually decreased to level of 34 mm.
9:09 p.m. Asphyxia: Small rise 5 mm. then decline to 0 (base line).

The above protocol shows well the condition of the cat. Just why it should have been in such a precarious state that the mere placing of it on its back was followed by the train of distressing symptoms cited above, is difficult to say. Apparently, compensatory mechanisms for maintaining blood pressure in emergencies were not operative, so that relatively slight exertion produced profound changes in internal conditions. Considerable though not complete anemia of the medulla probably occurred very soon after the cat began to struggle. It can be seen from the very slight rise in blood pressure on ligation of the cerebral arteries at 8:14 that function of the medulla had already ceased. Furthermore, it is clear that function was not restored by the higher level of pressure obtained on compression of the abdominal aorta at 8:25 since the subsequent ligation of the cerebral arteries at 8:47 was followed by no significant rise.

The case of cat 20 was somewhat different. Transection of the cord had been completed, the operation being attended by little hemorrhage. As the wound was being closed the respiration appeared to become somewhat irregular. The corneal reflex was present, as no ether had been given for some minutes. A few minutes later, however, the pupils were found to be widely dilated and the corneal reflex gone. At this time it was noticed that the cat was lying on the operating board in such a way that the abdomen sagged in consequence of lack of support. Respiration had failed. The cat was placed on its back and the thoracic walls compressed rhythmically. The impulse of the heart against the chest

wall showed the beat to be slow and weak. Warm saline was quickly injected into the stomach. There was no improvement in the condition. Tracheotomy was done, the thorax opened and artificial respiration was begun. The heart was flabby and beating weakly. The femoral vein was exposed and about 25 cc. of warm saline injected. The condition of the heart improved, the tone of the ventricles increasing and their action becoming more vigorous. The improvement was only temporary. A few drops of adrenalin were then tried. Again the heart was bolstered up for a few minutes only. Despite all remedial measures, there was no indication of real recovery.

The condition of the cardiovascular mechanism after an interval of recovery. After an interval varying from 2 to 32 days, the extent of recovery of systemic blood pressure was determined. The usual method of procedure was ether anesthesia; tracheotomy; preparation for occlusion of the head arteries and preparation for the stimulation of the brachial and the sciatic nerves and the spinal cord below the level of transection. Anemia of the brain was produced at will, by tightening ligatures placed around the left subclavian artery below the origin of the vertebral and around the right subclavian and carotid at their branching from the innominate (9). The cannula for blood pressure was placed in the left carotid artery in all but one instance and, in that case, the left carotid was also ligated. Artificial respiration was employed whenever it was necessary. It was always maintained during the period of occlusion of the head arteries. Twelve observations were made, including one cat with cord intact. Blood pressure was measured with a mercury manometer. Throughout the paper, the rise of blood pressure following occlusion of the head arteries is called the anemic rise, to distinguish it from the asphyxial rise tested out at the close of the experiment.

Amount of recovery of systemic pressure. The amount of recovery of blood pressure seemed to be influenced largely by the level of transection and, to some extent, by the length of time elapsing after the operation. Thus the average levels of blood pressure were 38 mm., 90 mm., 102 mm. and 114 mm. in cats transected at the level of the seventh cervical, first thoracic, third thoracic and fifth thoracic respectively. The periods of recovery in these cases were 6 days, 3 days, 10 and 32 respectively.

In the one rabbit in which the blood pressure was measured, the mean level was found to be 60 mm. Hg. No reflex vasomotor response was obtained on stimulation of the brachial or sciatic nerves. Slight variations in the level of blood pressure were produced by the stimulation of various nerves in the cervical region. No occlusion of the head arteries

or other means of analyzing the mechanism of the maintenance of this pressure was tried. The level of the transection of the spinal cord was in the region of the third thoracic segment.

The anemic rise of blood pressure and the pressure maintained by the isolated portion of the spinal cord. When a fairly constant level of blood pressure had been reached after the animal was anesthetized, anemia of the brain was produced by occlusion of the cerebral arteries. The immediate result of this procedure was a sharp rise—the anemic rise—which was usually interrupted by a temporary fall due to the inhibitory action of the vagus. The anemic effect again predominated, as shown by the continued rise in pressure. The pressure at the peak varied from 54 to 154 mm. A gradual fall then ensued and the constant level reached subsequent to this fall was taken as the blood pressure maintained by the spinal cord. The blood pressure curve is shown in figure 1.

The average spinal pressure obtained in these cats clearly indicated two things.

First: That the pressure obtained could scarcely be attributed to shock, since a comparison with cats with the spinal cord anatomically intact but with the medulla eliminated by anemia, or with cats in which transection of the cord had been done a few minutes before the measurement of blood pressure, shows that the pressures are nearly the same under all the various sets of conditions, or that the pressure is even lower in cats which have survived the transection several days. Judging by the usual criteria, one would expect the partial or complete subsidence of shock effects with the passing of time. Thus the average spinal pressure in the cats cited above was 26, 52, 48 and 35 mm. respectively and the period of recovery as stated before, was 6, 3, 10 and 32 days. The spinal pressure obtained in the cat with cord anatomically intact was 56 mm. This measurement is in line with results obtained by Stewart (10) and Pike (7), who found the average spinal pressure in a series of 60 or more normal cats to lie between 50 and 60 mm. Hg.

Second: That the isolated portion of the cord did not acquire any significantly greater influence in the recovery of the blood pressure and its maintenance with the passing of time up to the limit of life in this series of experiments. Thus a cat whose spinal cord was transected at the level of the first thoracic root showed a spinal pressure of 52 mm. after 3 days; another cat with the spinal cord transected at the level of the third thoracic segment had a spinal pressure of 40 mm. after 18 days; in a third cat in which the transection was made at the fifth thoracic root, a spinal pressure of 35 mm. was obtained after 32 days.

The effects of stimulation of the peripheral part of the spinal cord and the splanchnic nerves. It may be suggested that the low level of pressure maintained by the isolated portion of the cord, lower even than that found in normal cats upon occlusion of the cerebral arteries, was due to "inhibition" of the spinal cells of origin of efferent vasomotor fibers

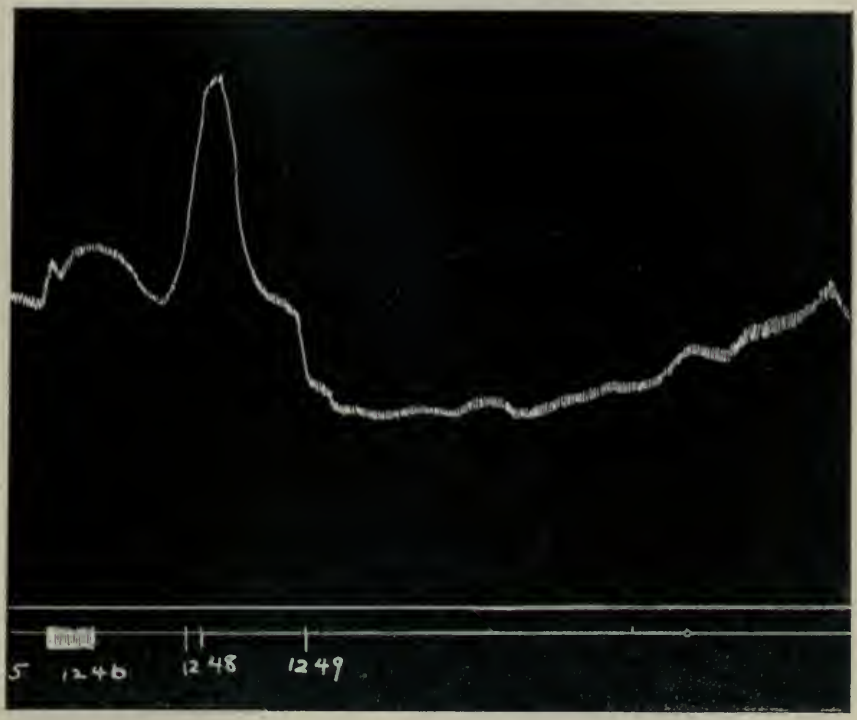

Fig. 1. Blood pressure curve of cat 13, showing blood pressure maintained before and after occlusion of the head arteries and the anemic rise. Head arteries occluded at 12:48 and released at 12:49. Transected at level of 1st thoracic 3 days before blood pressure was measured. The pressure rose after release of the head arteries until it reached the pre-occlusion level.

from the shock of transection. In order to test the validity of such an argument, the spinal cord was stimulated electrically just below the level of transection. An immediate and pronounced rise of blood pressure followed. That this electrical stimulus was greater than any natural stimulus, and for that reason might be able to pass the mysterious block imposed by shock, is a position not wholly tenable in view of the

fact that the mere placing of the electrodes on the cord was sufficient to produce a marked rise.

The muscular response which followed the stimulation of the cord was usually pronounced. There was extension of the hind limbs, spreading of the claws and, in one case, the tail was elevated and wagged violently. A rise of blood pressure was also obtained in the two cases in which branches of the right splanchnic nerve were stimulated.

Adequacy of the heart. It may be suggested that the consistently low blood pressure which was maintained in each case by the isolated portion of the spinal cord alone was due rather to an inadequate heart action than to the failure of the central vasomotor mechanism in this portion of the cord. That the low spinal pressure could not be laid to a weak heart action was shown by the great rise of pressure (12 mm. to 78 mm.) obtained on clamping the abdominal aorta. By constricting the aorta the peripheral resistance was considerably increased. If the heart were weak, even a great increase in peripheral resistance would not be effective in producing a marked rise of pressure. On the other hand, a strong heart alone cannot raise, or even maintain, the blood pressure at a normal level against the depressing effect of the widely dilated splanchnic vessels.

Effect of section of the nervi accelerantes or removal of the stellate ganglia. An estimation of the rôle of the nervi accelerantes in bringing about the rise of blood pressure resulting from anemia of the medulla oblongata could be reached only by a comparison of the anemic rise obtained when the extrinsic cardiac nervous mechanism was intact, with the anemic rise obtained after the section of the nervi accelerantes or the removal of the stellate ganglia. The difficulty of getting a recovery of the function of the medulla oblongata after the first occlusion in cats which had undergone complete transection of the cord, seriously hampered the acquisition of additional experimental data which it was hoped to gain for a further analysis of the mechanism of the anemic rise of blood pressure after transection of the spinal cord. First, the effect of occlusion of the head arteries with the extrinsic cardiac nerves intact was determined. In some cases however the effect of the vagus was so marked during the period of cerebral anemia that double vagotomy was advisable. In a few experiments it was possible to try a second anemic rise by ligation of the head arteries after section of the nervi accelerantes or excision of the stellate ganglia. It may be remarked in passing that in cats with spinal cord intact, several occlusions and recoveries are possible in the same animal.

It seemed likely that after transection of the spinal cord, the extrinsic cardiac nervous mechanism might be partly responsible for the anemic rise which follows occlusion of the head arteries. In three cats which showed an anemic rise of 74, 58 and 38 mm. with the stellates intact, a later occlusion of the head arteries resulted in only slight elevations of pressure—10, 6 and 6 mm. respectively. Where the spinal cord was intact, removal of the stellates had little effect on the rise of blood pressure. Occlusion of the head arteries was followed by a rise of 70 mm. in this case when the stellates were uninjured and by a rise of 140 mm. after their removal. The pre-occlusion level was lower after removal of

TABLE I

Showing the length of life of the animals and the blood pressure after spinal transection

NUMBER OF EXPERIMENT	LEVEL OF TRANSECTION	DAYS ANIMAL LIVED	AVERAGE SYSTEMIC BLOOD PRESSURE	AVERAGE SPINAL BLOOD PRESSURE	ANEMIC RISE ON OCCLUSION OF HEAD ARTERIES	
					Stellates intact	Stellates removed
			mm.	*mm.*	*mm.*	*mm.*
7a	Cord intact	0	120	56	70.0	140
6	VII C	5	62	38	74.0	10
18	VII C	6	38	26	6.0	
13	I T	3	90	52	58.0	6
3	II T	6	50	40	7, 8	
19	II T	2	56	34	10, 8	
4	III T	10	102	48	36	
11	III T	18	76, 90	40	38	6
15	III T	11	38	40	12	
2	V T	6				
5	V T	12	90	34	64	
14	V T	32	114	35		

the stellates than before, but the ultimate level of blood pressure after occlusion in these two instances was practically the same, 170 and 180 mm. respectively. Thus it seems that when all efferent vasomotor routes are open, as in the intact spinal cord, the reaction of the medulla against increasing anemia is shown by the sending out of vasoconstrictor impulses along the cord. Even when the cord is transected, the channels left open above the lesion are effective in compensating temporarily for the loss of blood to the medulla, but when, in addition to the transection, the stellates are removed, it is not possible to obtain so great a compensatory rise of pressure.

The general condition of the cardiovascular system as indicated by the measurement of blood pressure is shown in table 1.

Effect of stimulation of afferent nerves. It would be expected that stimulation of nerves such as the brachial and sciatic, would produce results in the cat with transected spinal cord different from those obtained in the normal cat. The level of transection might determine the extent of the response which the brachial would give on stimulation, i.e., if the level of transection were low enough to permit of a considerable efferent vasomotor outflow over the upper roots of the thoracic portion of the cord, the effect would be greater than if the transection were sufficiently high to interfere with this outflow.

In the case of the sciatic, one would expect a failure of its usual effect,—provided its afferent fibers actually do make a functional connection in the spinal cord with the cells of origin of the efferent vasomotor fibers—only when the level of transection passes so low in the cord as to fall below the segments from which these efferent fibers arise. But if the afferent fibers of the sciatic make their usual functional connection with the efferent fibers through the medulla oblongata, the transection of the cord should greatly decrease its usual effect on blood pressure. The stimulation of afferent nerves while the function of the medulla was intact and after its paralysis by anemia might be expected to yield some results of interest in relation to the rôle of the medulla oblongata and the spinal cord in the maintenance of blood pressure after spinal transection.

Stimulation of the brachial plexus and the sciatic nerve gave the following results before and after occlusion of the head arteries:

NUMBER OF EXPERIMENT	LEVEL OF TRANSECTION	BRACHIAL		SCIATIC	
		Before occlusion	After occlusion	Before occlusion	After occlusion
		mm.	*mm.*	*mm.*	*mm.*
18	VII C	2	0	−4	0
11	III T	20	1	10	2
15	III T	6	6	2	6
7a	Cord intact			28	0

No. 15, which gave such different results from no. 11, was in labor at the time the blood pressure was measured. Stimulation of the sciatic produced no significant rise before occlusion of the head arteries except in no. 7a. After occlusion, or failure of the medulla for some other reason, no significant rise occured except in one case in which an increase of 12 mm. was obtained. A rise of 8 mm. followed stimulation of the sciatic when the medulla oblongata of this animal was functional.

Effect of stimulation of the vagus. The effects of stimulation of the central end of the vagus deserve separate treatment because of the known anatomical nuclei of origin of its fibers, and its undoubted functional connections with the medulla oblongata. The result of stimulation of the vagus depended largely upon the state of the other vagus. When one vagus was intact, stimulation of the central end of the cut vagus when the medulla was functionally active brought about a considerable reflex fall, varying from 14 to 42 mm. In one case no reflex effect whatever was obtained, although stimulation of the intact vagus produced a great slowing of the heart and a marked fall of blood pressure. When both vagi were cut, stimulation of the central end of one usually had no effect on the blood pressure if the level of transection was high. In one case, however, a fall of 32 mm. was obtained under these conditions. All reflex responses of the vagus failed after paralysis of the medulla by occlusion of the head arteries.

In one cat a separate depressor nerve was found. Stimulation of the central end produced a marked fall of blood pressure.

Effect of asphyxia. It was found that on cessation of artifical respiration at the close of the experiment, and after complete functional elimination of the medulla, an asphyxial rise of blood pressure occurred in some animals. This rise was not always great, sometimes only 4 or 5 mm., though at other times it was as much as 14 or 18 mm. Usually this rise was followed by a gradual fall to base line as the heart became slower and weaker and finally stopped altogether. In two cases the initial rise and subsequent fall were followed by a second rise, one of 14 mm., another of 46 mm. The asphyxial rise was not greater in cats which had lived a considerable time after spinal transection than in cats which had lived only 2 or 3 days.

Pilomotor activity, frequently seen in asphyxiation, was observed in only one of the present series of animals (cat 19). Two minutes after the artificial respiration was stopped, the hind limbs and tail began to twitch. In a few moments more the hairs on the back and tail began to stand up.

Another effect of asphyxia is seen in the spontaneous evacuation of the bladder. This was noticed in two cats, 6 and 15, micturition occurring 9 minutes after the cessation of artificial respiration in one and 3 minutes after the beginning of asphyxia in the other. It is of interest, and possibly of some importance, that the second cat was pregnant and that the bladder had already acquired considerable automaticity during the interval of recovery. Spontaneous micturition has been observed by Pike.

during the asphyxial period, in cats with uninjured spinal cord, but with medulla paralyzed by anemia several hours previously. He also cites an instance of spontaneous emptying of the bladder, with active contraction of its muscular fibers, as much as 1 hour after the death of the cat. The abdominal wall had been opened soon after death, and the actual change in the size of the bladder was observed.

The duration of the heart beat after artificial respiration had been discontinued was found to be somewhat longer after the transection of the spinal cord than in cats with the cord intact. Thus in two normal cats the heart action continued 8 and 9 minutes after the beginning of the asphyxial period, whereas, in ten cats in which the spinal cord had been transected, the heart continued to beat from 8 to more than 23 minutes, the average being 12 to 15 minutes. There was no relation apparently between the level of transection and the duration of the heart beat in asphyxia.

Some incidental observations made during the course of the experiments. Certain incidental observations of interest in connection with the general functional condition of the spinal cord were made in addition to the results which were more directly connected with the main problem. The recovery of the heat-regulating mechanism, of the reflexes of the skeletal muscles, and the mechanism for micturition have already been discussed under the section on post-operative care.

Onset of rigor mortis. Upon arrival at the laboratory one morning at 8:30 o'clock, one cat was found dead from accidental strangulation. The body was already cold. However, it seemed worth while to try resuscitation, and within 3 minutes tracheotomy had been done and artificial respiration begun. The thorax was opened, the abdominal aorta clamped, and massage of the heart started. This was continued for about 40 minutes, but the only evidences of returning life were a few feeble flickers of the ventricles, so feeble indeed that at 9:15 first aid measures were abandoned and reluctantly the animal was pronounced beyond hope. At this time rigor mortis of the fore limbs was observed, though there was no post-mortem rigidity of the hind limbs. The condition of the fore limbs had not been noticed at the time tracheotomy was done. Five hours later rigor mortis was observed to have set in in the hind limbs. No other observations were made in the interval.

Ineffectual labor. One cat was in advanced pregnancy at the time of the operation. A good recovery was made. On the 10th day following the transection signs of labor were evident. There was considerable activity on the part of the foetuses. The old cat became quiet and

"dopey." The progress of labor was watched for 5 or 6 hours, but delivery did not occur. On the morning of the 11th day one fetus in breach presentation was found only partially delivered. Examination showed it to be dead. The other embryos were still alive and their movements in utero could be observed through the maternal body wall. Since it was only a matter of hours before septic processes would start in the mother cat, the blood pressure was measured at once.

The effect of intravenous injection of fluids. In a few cases where the blood pressure was very low, an effort was made to raise it by injecting warm saline into the femoral vein before ligating the head arteries. The saline was injected with a hypodermic syringe, each injection of 5 cc. being followed by a rise of 4 or 5 mm. The heart action was also greatly improved. This rise was not maintained, however. On one occasion a few drops of adrenalin were given with the saline. The customary sharp rise ensued but this too was transitory. The immediate response which the injection of even small quantities of saline produces in cats with transected cord is in direct contrast to the reaction of those where spinal cords are intact. In the latter the level of blood pressure is not appreciably affected until large quantities of fluid have been injected. The reaction to the intravenous injection of fluids in some animals whose spinal cord was transected but whose medulla oblongata was intact, was essentially that of a purely spinal animal. The medulla was exerting little or no influence on the blood pressure.

Prominence of the nictitating membrane. In cats in which the level of transection was high—seventh cervical or first thoracic segment—the nictitating membrane was more than usually prominent, being drawn well toward the pupil. This is probably due to injury to the fibers of the sympathetic system which emerge from the spinal cord in the upper thoracic segment, pass through the stellate ganglion, and up to the eye.

THE GENERAL THEORETICAL SIGNIFICANCE AND THE RELATIONSHIPS OF THE EXPERIMENTAL RESULTS

Mechanism of recovery or maintenance of systemic blood pressure after spinal transection: The amount of recovery of blood pressure. If the blood pressure is measured some time after transection of the cord, it is not possible to ascertain from such measurement alone the factors which are operating to maintain the level of pressure found. Since this is so, it becomes necessary to resort to some other experimental procedure which will result in a further analysis of these factors. Any conclusion

regarding the mechanism for the recovery or the maintenance of blood pressure must be based on the experimental results obtained. Therefore it is necessary to consider the bearing of the results on this problem.

The exact amount of recovery of blood pressure is difficult to determine. The literature contains no systematic information as to the average blood pressure after transection of the cord at different levels. However, two lines of evidence present themselves as criteria on which to base the assumption that the blood pressure is higher after an interval of recovery than immediately after the transverse lesion is made. These are the unpublished experiments of C. S. Winkin (11) done in the Columbia laboratory and the condition of the animals in my series observed daily from the time of operation until the measurement of blood pressure was made.

Mrs. Winkin found a significantly lower pressure in cats in which the lesion was made in the region of the second and third thoracic segments than was observed in the present series of experiments where the blood pressure was measured some days after the transection. Furthermore, in my series, immediately after the operation, the animals gave every indication of weakness. They showed no inclination to move about but lay quietly in the pan. The body temperature was subnormal. They manifested no interest in food. Within a day or so the picture changed. They had become sufficiently active so that confinement in a cage was necessary. The body temperature rose and food was consumed with apparent relish. It is also significant that the cats with high transection showed very little recovery in the post-operative period—in fact, several died—and the level of blood pressure in those that lived was low. In every case of unusually low blood pressure before anemia of the brain, there were concomitant manifestations of indisposition.

Evidence for central mechanism. The precise extent of recovery is more or less immaterial—even the question as to whether or not any recovery has occurred is, from the point of view of these experiments, of secondary importance. That a certain level of blood pressure is being maintained after transection of the cord and that this pressure is ultimately sufficient for the performance of the usual metabolic processes is indubitable. The matter of prime importance is the central mechanism which is responsible for the maintenance of this pressure, whatever it may be. Does it lie in the medulla oblongata or is it to be found in the cells of origin in the spinal cord?

The evidence for the location of the central mechanism in the medulla lies in the results obtained by ligation of the head arteries, by stimula-

lation of certain afferent nerves before and during cerebral anemia, and in the effect of excision of the stellate ganglia or section of the extrinsic cardiac nerves before and after occlusion of the head arteries.

It is obvious that the occurrence of a significant rise of blood pressure on ligation of the head arteries is undoubted evidence that the medulla oblongata is able to affect either the rate of the heart or the caliber of the blood vessels, or both. Control experiments have shown that the mere mechanical effect of shutting off the circulation to the head, in the absence of any functional activity of the medulla, is to bring about only a trifling rise of blood pressure. Other control experiments have shown that the change in the caliber of the blood vessels is the most important agent in bringing about this rise. For it occurs in undiminished intensity when the extrinsic cardiac nerves are divided and the vasomotor nerves intact. (See cat 7 a.) Also the rise is small when the cardiac nerves or the nervi accelerantes, at least, are intact, but the thoracic ganglionic chain divided at the level of the diaphragm. (Unpublished results of Mrs. C. S. Winkin.) The occurrence of the usual anemic rise of blood pressure in cats some days after complete transection of the spinal cord is strong evidence, therefore, that the central vasomotor mechanism in the medulla oblongata can exert some influence upon the caliber of the splanchnic blood vessels. This is in direct contradiction to Goltz's statement, for which he gave no experimental proof, that the portion of the nervous system lying above the level of transection had nothing to do with the recovery or maintenance of blood pressure in the region below the level of the spinal transection.

Effect of stimulation of afferent nerves. Stimulation of several strands of the brachial plexus before functional elimination of the medulla resulted in a rise of pressure, which disappeared with the cessation of bulbar function. It may be said that the failure of the usual vasomotor response of the brachial nerves was due to a "diaschisis" effect on the cells of origin located in the cervical cord following paralysis of the bulb. But even if such a diaschisis effect is possible—though far from probable—the mere occurrence of a reflex vasomotor response on stimulation of the brachial would indicate that some mechanism lying above the level of transection was exerting some influence on systemic blood pressure.

A fall in blood pressure on stimulation of the central end of one vagus, the other being intact, may be brought about in two ways:—*a*, by the cardio-inhibitory action of the intact vagus, or *b*, as a result of stimulation of depressor fibers which accompany the vagus in the cat. The inhibi-

tory action of the intact vagus was probably the cause of the fall in the majority of cases herein cited. But the fall in pressure which was obtained by stimulation of the central end of one vagus after both had been sectioned may be explained by the action of the depressor. The effect of the depressor, however, involves an action upon the blood vessels of the splanchnic region. The evidence obtained from stimulation of the central end of the vagus points in the same direction as that obtained from stimulation of the brachial. But in the case of the vagus, its known anatomical relations indicate that the reflex pathway passes through the medulla oblongata, and the complete failure of the response after paralysis of the medulla reveals the necessity of a central connection through the medulla, and the prime importance of the bulbar mechanism in the control of cardio-vascular reactions.

Effect of excision of the stellate ganglia or section of the nervi accelerantes. It is apparent from the evidence presented that the vasomotor mechanism is a more powerful agent in bringing about changes of systemic blood pressure, under most conditions at least, than the extrinsic cardiac nervous mechanism. But the rôle of the extrinsic cardiac nerves and particularly the accelerators in compensating for changes in the caliber of the blood vessels comes out prominently under other experimental conditions. The failure to obtain a large rise of blood pressure on occlusion of the head arteries of the cats with transected spinal cords after section of the accelerators or excision of the stellate ganglia calls attention to the rôle of the accelerators. In the case of excision of the stellates, the failure of the usual rise on occlusion of the head arteries might be due to the interruption of the nervi accelerantes or to the interruption of vasomotor fibers passing through the stellate ganglia. It seems probable that the nervi accelerantes are an important part of this compensatory mechanism since merely cutting all cardiac branches from the stellates results in a failure to obtain a rise.

The resemblances or differences in deportment of animals with intact extrinsic cardiac nerves, but with transected spinal cord and impaired vasomotor system, as compared with animals with intact spinal cord and vasomotors but with division of the extrinsic cardiac nerves, can be extended still farther. Animals in which the extrinsic cardiac nerves have been divided exhibit no discomfort when at rest, but are unable to undergo any considerable exertion (12). One sometimes sees in the textbooks (13) the statement that the splanchnic vessels contract while the peripheral vessels to the somatic musculature dilate on muscular exertion. The facts brought out in this paper show that the splanch-

nic blood vessels may, by the narrowing of their lumen, bring about a great rise of blood pressure. If any significant amount of contraction of the walls of the splanchnic vessels actually occurred, one would expect a sufficiently high blood pressure to permit an animal deprived of its cardiac regulatory nerves to maintain a considerable degree of activity. The question apparently needs further study.

The effect of asphyxiation: The mechanism of the asphyxial rise. The asphyxial rise of blood pressure occurring at the close of the experiment is of considerable interest. To what can this asphyxial rise be attributed? Obviously, it must be due to an increased heart action or to vasoconstriction, in response to the accumulation of carbon dioxide in the blood. Examination of the records reveals no increase in heart action either in rate or force. Indeed, the beat becomes progressively weaker. The vasomotor mechanism, then, must be responsible for the effect. The vasoconstrictor center had been paralyzed for some time; therefore it is hardly conceivable that it could be further affected by the excess of CO_2 in the general circulation. This leaves the possibility of stimulation of cells of origin of efferent vasomotor fibers in the spinal cord or of direct stimulation of the vascular musculature. Apocodeine administered intravenously (14) abolishes the asphyxial rise. According to Dixon, apocodeine paralyzes central cells in the spinal cord. In view of these facts it appears that the asphyxial rise of blood pressure on cessation of artificial respiration is due to the stimulation of the spinal cells of origin of vasomotor fibers by carbon dioxide. Here again we find cells in the isolated portion of the spinal cord responding to a stimulus and this a "natural" stimulus. Could they react in this way if they were really inhibited by shock? On the contrary this response furnishes additional evidence of the influence of the bulbar centers since the cells of the spinal cord are still fully able to function. The results so far obtained indicate that the activity of these spinal cells is dependent on nerve impulses from higher centers. Nor apparently do they discharge efferent impulses over vasomotor fibers in response to afferent impulses from outside the central nervous system. Usual spinal reflex arcs are open and functional, e.g., stimulation of the sciatic produced a marked muscular contraction of the hind leg, sometimes of both hind legs. If, then, afferent pathways from the viscera to the spinal vasomotor cells existed, it would not be unreasonable to suppose that vasomotor changes could be brought about and blood pressure maintained through reflex action involving only the lower centers.

The duration of the heart beat. The duration of the heart beat in asphyxia in all probability bears a direct relationship to the rapidity of accumulation of carbon dioxide in the blood, and this, in turn, is related to the degree of activity of the skeletal muscles. It is known that the duration of the heart beat in asphyxia after paralysis of the skeletal muscles by curare is much greater than when no curare has been given, extending at times over a period of an hour. The significantly longer duration of the heart beat in asphyxia in the cats of this series as compared with normal cats indicates a decreased activity of the skeletal muscles. The relation of length of the period of recovery after the first operation to the duration of the heart beat in asphyxia is not clear from this series of experiments.

Rigor mortis. The observations on the duration of the heart beat in asphyxia are in line with the delayed onset of rigor mortis in the hind limbs after spinal transection. Both series of facts indicate a decreased activity of the skeletal muscles under these conditions.

Incidental observations: Ineffectual labor. Goltz (15) cites two instances in which labor ran its normal course after transection of the spinal cord. In the first case conception, pregnancy and parturition occurred after the cord had been cut at the level of the first lumbar vertebra. Nearly 8 months later the dog gave birth to one live puppy. Two dead fetuses were removed and the bitch died 2 days later of peritonitis. In the second case the cord was transected at the level of the third thoracic vertebra and 3 days later 94 mm. of the lumbar cord were removed. Four hours after this second operation, the first of five live puppies was delivered. The duration of labor was about 30 hours. The bitch lived nearly 2 months after the birth of the pups. The cause of the failure of a normal delivery in the case of the cat, 10 days after spinal transection at the level of the third thoracic vertebra, is not clear.

Certain aspects of the relation of the nervous mechanism to the regulation of physico-chemical conditions in the animal organism upon which these results have a bearing may be indicated here. Because of lack of time the observations were not made during the course of the experiments, but the problems may be attacked more specifically at some future time. It is commonly stated that the secretion of urine ceases when the systemic blood pressure falls below 30 or 40 mm. Hg. This raises the question whether the death of some of the animals with transection of the spinal cord at the level of the seventh cervical segment may not have been due to uremia, due to lack of secretion of urine. In a few instances, the bladder was found practically empty at autopsy, but

usually there was sufficient urine to allow of its expression during the life of the animal. No analyses of blood were made in this connection, and therefore no definite answer to the question can be given now. The known relation of low systemic blood pressure to a low concentration of carbon dioxide in the blood raises the further question as to the concentration of carbon dioxide in the blood of the animals used in this research during the period of recovery. No specific observations were made, and the answer to the question must be deferred until further experiments are done. Further observations of this nature are needed to bring out the relationship of the nervous system to the physico-chemical regulation of the organism. If, as there is every reason to suppose, the law of mass action applies to many of the reactions in the living organism, any lesion of the nervous system which modifies in any way the conditions of pressure in the organism might be expected to have its effect on some metabolic process (16). The results now in the literature are sufficient to indicate that this possibility is, in some instances at least, a reality.

CONCLUSIONS

1. Recovery of systemic blood pressure occurs after an interval in cats in which there has been complete transection of the spinal cord, if the level of transection does not lie above the level of outflow of the thoracic sympathetic chain.

2. The extent of the recovery of blood pressure depends largely upon the level at which the transection is made and partly upon the length of time allowed for recovery. In a few cases the recovery was nearly complete.

3. The mechanism through which the recovery is brought about includes the cardio-vascular mechanism in the medulla, and the outflow from the center which includes the extrinsic cardiac nerves and the vasomotor nerves. The vasomotor portion of this outflow is of the greater importance.

4. Spinal cells of origin of efferent vasomotor fibers lying below the level of transection do not take on, within the time limits of these experiments—2 days to 32 days—any significant part of the burden of maintaining the systemic blood pressure.

5. The experimental results lend no support to the view that there are spinal vasomotor centers functionally active and coördinate in rank with the mechanism in the medulla oblongata. They do support the

view that the bulbar vasomotor mechanism is the chief mechanism upon which the functional activity of all other parts of the vasomotor system depends.

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THE EFFECT OF HEMORRHAGE ON THE SYMPATHETIC NERVES

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Received for publication May 16, 1921

We have observed, as others (1) have, that hemorrhage not only produces hyperglycemia, but also that it sensitizes or stimulates the sympathetics as judged by the response to injections of adrenalin or nicotine. This is shown by the greater blood pressure rise, caused by the injection of a constant amount of adrenalin or nicotine before and after hemorrhage. The present investigation is an attempt to analyze the effect of hemorrhage on the nervous system, and to determine whether the action is central or peripheral, or on both locations; and secondly, to determine whether the increase in blood sugar is the only result of sympathetic stimulation or whether the hyperglycemia in turn influences the sympathetics.[1]

The physical changes in the vasomotor response have been investigated especially by Porter (2) and by Sollmann (3), but they do not agree on interpretation. Porter thinks the percentile rise in blood pressure is the true measure of the vasomotor response, while Sollmann and Pilcher say that the absolute rise is the more correct index. Neither method is wholly satisfactory and neither investigator analyzes the cause of the rise in blood pressure, but assumes it to be an action on the center. In this and similar work, the vasomotor response cannot be correctly interpreted as an action on the vasomotor center, but merely on the vasomotor mechanism, and the peripheral part of this mechanism may be more important than the central. Adrenalin, for example, raises the blood pressure but it has practically no influence on the vasomotor center. In the case of hemorrhage in particular we think most of the influence is peripheral. The present work supports this opinion. However, it is highly improbable that any action on nerves is confined absolutely to one location, though it may be mainly local. For example,

[1] The relation of increased sugar and epinephrin and nicotine action is being investigated by Drs. P. G. Albrecht and J. T. Groot.

while the action of curara is mainly peripheral it has also a central action (4), and while strychnine acts mainly on the cord, it may also have a peripheral action; so also the effect of hemorrhage on the sympathetics can hardly be expected to be confined absolutely to the periphery although its greatest action is there. Here again much work on the vasomotor center is needlessly complicated by the use of curara, and while Sollmann (3) justifies its use in some cases he found that it acted on the vasomotor center. McGuigan (4) has also shown that curara has a central action, and Reid Hunt (5) found after large doses of curara a reflex pressure rise may be absent.

That the effect of hemorrhage on the sympathetics is mainly peripheral, however, we think is supported by the following facts and experiments:

During anesthesia the rise in blood pressure after adrenalin is less than if no anesthetic had been given, and anesthetics depress the centers mainly (cf. with strychnine which stimulates these centers).

This might be taken to prove that the greater action of adrenalin after hemorrhage is due to central action. However such an assumption ignores the important fact that anesthetics depress also the peripheral mechansim. It is obligatory, therefore, in the analysis of this problem to stimulate or depress the centers independently of the peripheral mechanism. This we have done.

We have a method of stimulating the central parts of the nervous system without directly involving the peripheral parts. It is well known that many drugs injected into the cerebrospinal fluid are but slowly absorbed and therefore, for a long time, act locally. Taking advantage of this fact, we can stimulate the central system with strychnine, caffeine, cocaine, etc., or depress it by chloral, chloroform, etc., independently of the peripheral parts. In such cases it is found that when the centers are highly sensitized, the effect of adrenalin is no greater than before and that depression of the centers also has little influence.

The following experiment is illustrative:

Dog 8 kilos—1 cc. adrenalin 1:10,000 intravenously.

Normal blood pressure	*Blood pressure after adrenalin*
160 mm. Hg.	225 mm. Hg. rise—65 mm. Hg.
160 mm. Hg.	230 mm. Hg. rise—70 mm. Hg.

Strychnine was now injected into the 4th ventricle until a definite twitching of the head was elicited. The blood pressure was now—

Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.
Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.
Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.

Similar results are obtained whether the vagi are cut or intact.

Here it is seen that stimulation of the anterior part of the central nervous system causes no increase in the effect of adrenalin or even lessens the effect. The charge may be made legitimately, that strychnine in this region does not stimulate part of the cord from which the major part of the sympathetic arises. This may be true, and to make the test complete, we have strychninized the region below the medulla with the following result:

A dog 10 kilos—under ether had a blood pressure of 110 mm. Hg. After 50 cc. hemorrhage this rapidly dropped to 40 mm. Hg.; 1 cc. 1:10,000 adrenalin now caused a rise of blood pressure to 140 mm. Hg. or 100 mm. rise. The blood was defibrinated and most of it was injected into the femoral vein. This raised the blood pressure to 80 mm. Hg. One cubic centimeter adrenalin (1:10,000) now raised the pressure to 165 mm. or a rise of 85 mm. Hg. This however soon fell to 40 mm. again. At this point the sympathetic region of the animal (dorsal) was strychninized until definite twitching in this region occurred. One cubic centimeter adrenalin now caused a rise of blood pressure to 70 mm. Hg. Apparently, therefore, central strychninization does not increase vasomotor response to adrenalin.

Another experiment with the whole animal under the influence of strychnine, shows that central stimulation with adrenalin does not increase the pressure.

Dog 15 kilos—ether anesthesia. Normal blood pressure 100 mm. Hg. The same volume of adrenalin was used in all cases. One cubic centimeter 1:10,000 adrenalin intravenously raised blood pressure to 145 mm. or a rise of 45 mm. Hg.

Strychnine was now given until definite twitching occurred after which three separate injections of 1 cc. adrenalin raised the pressure to 145 mm. Hg. or an average rise of 45 mm. Hg. There was no spasm from the strychnine, but merely twitching. The animal was now bled 100 cc., this lowered pressure to 90 mm.

Adrenalin now raised it to 130 mm.
Adrenalin now raised it to 140 mm. or
An average rise of 45 mm.

Another hemorrhage of 150 cc. lowered the pressure to 55 mm. Hg.

Adrenalin now raised pressure to 105 or 55 mm. rise. The animal was again bled 80 cc. This lowered the pressure to 35 mm. Adrenalin now raised the pressure to 78 or 43 mm. Hg. rise. In this experiment adrenalin caused the same rise after strychnine as before. In other cases we have found a lesser rise after strychninization. Hemorrhage in this case again caused a sensitization to adrenalin action. Since the centers were apparently stimulated to the maximal by strychnine the action of hemorrhage seems to be peripheral.

As is well known, stimulation of the central end of a peripheral nerve raises the blood pressure. This is taken as an action on the vasomotor

center. The degree of rise depends on the condition of the center. If, therefore, we stimulate or depress the center, we vary the response to sciatic stimulation accordingly. If, then, the condition of the central nervous system changes the vasomotor response to those influences which we know act on the center and does not change the response to adrenalin, we are justified in assuming that adrenalin does not act on the center. In addition, if adrenalin is more effective after hemorrhage we are justified also in assuming that hemorrhage acts peripherally on the nervous system. However, when the region of the fourth ventricle is fully strychninized stimulation of the sciatic causes no greater rise of blood pressure than before, unless some tetanus occurs. This means that the vasomotor mechanism is but little acted on by strychnine and the method does not enable us to settle the question.

The effect of cocaine on the cord. It is well known that the action of cocaine and epinephrin are synergistic. Such synergism is manifestly proven only for the periphery. Since cocaine acts centrally also, it may be assumed that if adrenalin acts centrally there is also a central synergism; but if sufficient cocaine be given to eliminate conduction in the cord, this would manifestly eliminate all possible synergistic actions.

To positively place cocaine in the cord in several cases we laid it bare by operation. In addition to permitting the placing of cocaine on the cord the operation in this case had the desirable effect of lowering the blood pressure. This can be considered as a hemorrhage into the blood vessles. We have therefore the effect of hemorrhage and the removal of central influence. Under such conditions the central action of cocaine does not change the response to adrenalin. We can, therefore, assume that hemorrhage in this case acts more peripherally than centrally. The following experiment shows the effect of hemorrhage and cocaine. All hemorrhages, however do not give an absolute blood pressure increase to adrenalin but all do give a percentile increase; many give the same percentile increase and some a greater absolute increase.

Dog 10 kilos. Tracheal cannula, vagi cut. Adrenalin, 1 cc. at each injection 1:20,000. Pressure expressed in mm. Hg.

Normal pressure 146. Adrenalin raises it to 210, 206, 210, average rise = 63.
After 60 cc. hemorrhage—the pressure is..... 135
Adrenalin now raises pressure to..... 202, rise = 67
After another hemorrhage of 45 cc. pressure..... 114
Adrenalin raises to..... 196 or 82 rise
5 cc. 0.5 per cent cocaine in 4th ventricle stops respiration and reduces pressure to..... 82
1 cc. adrenalin now raised pressure to..... 156 or 74 rise
Cocaine 5 cc. intravenously pressure now..... 116
Adrenalin raises pressure to..... 226 or 110 rise

The hemorrhage here lowers the blood pressure, but the adrenalin is more effective, and this means stimulation of the vasomotor mechanism. Stimulating the center with cocaine does not increase effectiveness of adrenalin, but the peripheral action of cocaine enormously increases the effectiveness. We think, therefore, since the condition of the center seems not to change the vasomotor response, that the effect of hemorrhage is peripheral.

I. Action of nicotine

Young dog—weight 5 kilos—vagi cut—tracheal cannula for ether. 1 cc. 1:2,000 adrenalin used in each case. Normal blood pressure 70.

Rise with adrenalin—58—in three cases. Nicotine was now given intravenously until no blood pressure rise was obtained on last injection.

The blood pressure is..... 78

Adrenalin now raises pressure in 2 administrations, 94 and 100 or an average rise of..... 97

which is almost twice the normal rise before nicotine.

This would indicate that the central effect of adrenalin, if any, works against a rise of pressure, and favors the view that the action of adrenalin after hemorrhage is peripheral.

II. Action of nicotine

Dog—weight 15 kilos. This animal had 6 grams of chloral hydrate 4 hours previously. Adrenalin used 1:20,000. Tracheal cannula inserted for anesthesia, and vagi are cut.

Normal blood pressure..... 176

Rise by 1 cc. adrenalin..... 48

After paralysis of the vasomotors by nicotine, 1 cc. adrenalin caused a rise of 84 or almost twice the normal rise.

III. Action of nicotine

Dog—weight 14 kilos. Nicotine 1–4000, was used intravenously, since paralysis of the ganglions was not desired. Carotid pressure—vagi cut—ether anesthesia.

Normal blood pressure..... 204

1 cc. nicotine raises pressure to..... 210 or a rise of 6

60 cc. blood withdrawn, reduces pressure to..... 182

1 cc. nicotine now raised it to..... 198 or a rise of 16

93 cc. blood withdrawn, lowers pressure to..... 156

Nicotine now raises it to..... 198 or a rise of 42

IV. Action of chloral centrally

Dog—weight 12 kilos. Nitrous oxide anesthesia—no ether used. Vagi cut. Adrenalin used 1:40,000, 1 cc. at each injection. Normal pressure 192.

Adrenalin raises to 252 or a rise of 60.

Bled 100 cc. which lowered pressure to 156.

Adrenalin raises pressure to 218 or a rise of 62.
Adrenalin raises pressure to 222 or a rise of 66.
4 cc. chloral hydrate (2 per cent) into 4th ventricle after which the pressure is 168.
Adrenalin raises pressure to 230 or a rise of 62.
Animal bled again—100 cc. which lowered pressure to 100.
Adrenalin raises to 158 or a rise of 58.

Summary. The effect of chloral into the 4th ventricle sufficient to anesthetize the animal deeply, is without apparent effect on the reaction to adrenalin.

V. Action of chloral

May 12, 1921. Dog weight—10 kilos—no ether or vol. anesthetic. Time: 1:42; 5 cc. 10 per cent chloral hydrate into 4th ventricle gives immediate anesthesia. This was the only anesthetic used.
At 1:50: Tracheal carotid and femoral cannulas inserted and vagi cut.
Adrenalin used 1 cc. 1:80,000 at each injection.

Normal rise caused by adrenalin	93

Normal presure 95, but it remains at 126 after three injections of adrenalin.

Bled 58 cc. reduces pressure to	118
Adrenalin now raises pressure to	222
or a rise of	104
Bled 80 cc. reduces pressure to	108
Adrenalin now raises the pressure to	216
or a rise of	108
Further bleeding of 100 cc. reduces the pressure to	62
Adrenalin now raises pressure	128
or a rise of	66

In this case, the nerve endings are not anesthetized and after hemorrhage adrenalin causes a greater rise, until the hemorrhage is so great that the vessels are too flaccid to give an increase rise. Even then the percentile rise is greater than the normal. Such cases as this render it unnecessary to discuss whether the percentile or absolute rise is the proper method of interpretation, since both are increased.

DISCUSSION

The present investigation was suggested from work on the influence of the nervous system on the blood sugar. In that work it was found that all nervous impulses that increase the sugar in the blood pass over the sympathetic nervous system. Others have also reached this conclusion. The proof of this is briefly: stimulation of the motor areas does not increase blood sugar. Piqûre does not increase it if the splanchnic nerves have been severed; stimulation of sensory nerves may or may not give a hyperglycemia; similarly, neuritis of various forms is only

rarely accompanied by hyperglycemia. Nicotine does not increase blood sugar after adrenalin; in other words, stimulation of the parasympathetic ganglions only has no effect; similarly pilocarpine and atropine are without significant influence on the blood sugar (6). Therefore, the sympathetics are the important nerves in blood sugar regulation.

Since hemorrhage also causes hyperglycemia it seems that it stimulates the sympathetics, and since strychnine may be used to free the liver of glycogen its use in the present problem was suggested as probably also acting on the sympathetics.

The objection may be made that strychnine sensitization of the cord does not involve the sympathetics. However in addition to the above citations there is a considerable amount of evidence that it does. Hefftter (7) cites proof of an action on the vegetative system and it is well known that strychnine will free the glycogen from the liver, and since all impulses increasing the blood sugar pass over the sympathetics strychnine may be correctly used to sensitize the central nervous system in the present problem. The method is especially justified since strychnine in the doses used strongly stimulates the vasomotor center (4), and has very little peripheral effect.

Hemorrhage might conceivably increase the secretion of adrenalin into the blood and at least partly account for the increased irritability. However, Hoskins and Rowley (8) found that no concentration of adrenalin injected into the blood augments vasomotor irritability, and sometimes lessens it. This depression they think is probably both central and peripheral. We have noticed, however, that the first, sometimes also the second and third injection of 1 cc. 1: 20,000 adrenalin solution is less effective in raising the blood pressure in anesthetized animals than in succeeding injections, which may mean some sensitization. With Rosser, Hoskins and Rowley (1) studied the effect of hemorrhage on vasomotor irritability, and found it increased as judged by the response to nicotine. The increase they think is confined to the vasomotor center. It is questionable, however, whether the method used is adequate. They used adrenalin, the concentration (1:100,000) which is perhaps too weak to indicate small changes; with this concentration they found no increased irritability in the animals which were either under ether anesthesia or decerebrate. In the latter group the low concentration of adrenalin used would be much more effective. It is well known that in chemical titrations some indicators are not sufficiently delicate to show considerable changes in the concentration of hydrogen ions, so adrenalin in this dilution may be inadequate to indicate a change in this case.

With nicotine they obtained positive results which we can confirm, and since the results (peripheral) with adrenalin were negative, they conclude the positive effect of nicotine was probably due to increased central irritability. However, while nicotine (4) stimulates the vasomotor center the main action is on peripheral ganglion cells and the effect recorded in these experiments was apparently on the sympathetic ganglia and, therefore, peripheral and in agreement with our own.

By measuring the rise of blood pressure on stimulation of the central end of the sciatic or brachial nerve, Porter (9) has shown that the tone of the vasomotor center is little affected by hemorrhage. In arriving at this conclusion he lays stress on the percentile rather than the absolute rise, as the true index of the condition of the vasomotor cells. When the percentile rise is taken as the index there is a stimulation of the vasomotor center. This fact if accepted has little bearing on the present problem except in confirming the opinion of Hoskins (8). Sollmann states that the absolute rise is a more reliable criterion of the response of the vasomotor center than is the percentile rise, and recently Crile (10) has pointed out the weakness of Porter's logic. Stimulation of a peripheral nerve, however, as admitted by all who have used it, is an unsatisfactory method of determining the condition of the center. In the present investigation we have to a great degree eliminated the necessity of discussion over the relative values of percentile and absolute changes, since in many cases both are changed.

If we look at blood pressure as we do at balanced reaction which tends to reach an equilibrium at a definite height in the reaction, we should expect a greater percentile rise when the pressure is low. If this is not permissible, it certainly is obvious that the same force exerted at a low pressure should be more effective in raising the pressure than if it were exerted at a higher level. Now *ceteris paribus*, stimulation of the vasomotor center should increase the effectiveness of adrenalin on the blood pressure, because it is known that adrenalin is more effective without anesthesia, which depresses the centers; but great weight cannot be given to this because a general anesthetic also depresses the vasomotor endings which is the main point of action of adrenalin. While adrenalin may act on the center directly such an action can be shown only with especial technique (11), (12), (13), (14) and from the results cited we think the central stimulation after hemorrhage is of less importance than the peripheral effect. The peripheral action of adrenalin seems less in many cases after strychnine and other central stimulating drugs. This may be similar to the action of the brain on the lower motor neu-

rons, i.e., the influence of the center restrains peripheral action. Similarly after nicotine, which removes all central influence, adrenalin is a more powerful vasoconstrictor.

SUMMARY

Hemorrhage in many cases sensitizes or stimulates the sympathetic system governing vascular tone. By the use of drugs that are known to stimulate or depress the centers and which have a lesser or no effect on the periphery, it is shown that the greater influence of the hemorrhage on the vasomotor mechanism is peripheral.

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(7) Handb. d. Exper. Pharm., 1920, ii, 362.
(8) Hoskins and Rowley: This Journal, 1915, xxxvii, 480.
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THE CHANGES IN THE CONCENTRATION OF THE CARBON DIOXIDE RESULTING FROM CHANGES IN THE VOLUME OF BLOOD FLOWING THROUGH THE MEDULLA OBLONGATA

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Received for publication May 18, 1921

Attention has been called to the changes in respiration in dogs following the permanent ligation of the two carotid and two vertebral arteries as in Sir Astley Cooper's experiments (1), (2). A question asked by a friend as to the effect of changes in respiration consequent upon changes in the volume of blood flowing through the medulla oblongata led to the repetition of these experiments in a modified form, with cats as the experimental animals, and temporary instead of permanent ligation of the arteries, and the estimation of the carbon dioxide in the arterial blood under the various conditions of blood flow through the medulla. A preliminary note has been published, giving some of these changes in the gases of the blood (3).

The experiments reported here were made on cats. Ether and tracheotomy were routine procedures. Blood pressure was taken by a mercury manometer connected with one carotid artery. The other carotid was cleaned from its surrounding tissues and loosely ligated. The two vertebral arteries were isolated before their entrance into the foramina of the transverse processes of the cervical vertebrae and loosely ligated. Arterial blood for analysis was drawn from one femoral artery and centrifuged without exposure to the air. The carbon dioxide content of the plasma was determined by the Van Slyke method. The respiratory movements were recorded by a Crile stethograph connected with a Verdin tambour. Samples of blood were taken for analysis before placing clamps on the arteries, while the clamps were on, and after their removal. Usually 10 cc. of blood were drawn at one time.

The effect of temporarily clamping the four arteries is an increase in magnitude or rate of the respiratory movements, and a marked rise of the arterial blood pressure. During the period of increased respira-

tory activity, the concentration of carbon dioxide falls in the arterial blood. (Tables 1 and 2.) When the flow was long obstructed there was, of course, an accumulation of carbon dioxide in the blood as the second sample in table 2 indicates. When the circulation to the head is restored, the blood pressure promptly returns to normal, and the

TABLE 1

DATE	NUMBER OF OCCLUSIONS	AVERAGE CO_2 CONTENT OF PLASMA IN VOLUMES PER CENT	
		Before occlusion	After occlusion
1- 6-19	4	31.1	30.4
1- 8-19	4	46.9	41.4
1-10-19	4	46.8	43.0
12-18-18	2	55.0	45.0
12-21-18	2	41.0	38.0
12-26-18	4	49.8	44.7

Only samples obtained before 40 per cent of the blood had been drawn are included in this table.

TABLE 2

Experiment 1-10-19

NUMBER OF SAMPLE	CO_2 CONTENT OF PLASMA IN	EXPERIMENTAL PROCEDURE	NUMBER OF SAMPLE	CO_2 CONTENT OF PLASMA IN	EXPERIMENTAL PROCEDURE
	volumes per cent			*volumes per cent*	
1	49.0	Anæsthesia	9	46.0	Obstruction continued
2	52.5	Flow long obstructed	10	44.7	Free flow; 44 per cent blood drawn
3	48.5	Free flow	11	38.0	Flow obstructed
4	46.0	Obstruction to flow	12	35.0	Free flow
5	48.0	Free flow	13	31.1	Flow obstructed
6	43.5	Obstruction to flow	14	31.5	Obstruction continued
7	46.0	Free flow	15	24.7	Flow obstructed
8	44.5	Obstruction to flow	16	24.7	Free flow

respiratory movements resume their usual rate and amplitude, or closely approach normal.

Occasionally clamping all four arteries results in too great restriction of blood flow to the medulla oblongata, and the first great increase in respiratory movements is soon followed by a slowing or stoppage. In such cases, one vertebral artery may be released. As successive samples

of blood are withdrawn for analysis, resulting in a diminution of the volume of the circulating fluid, closure of one carotid artery, leaving both vertebrals open, may be sufficient to produce the desired change in respiration.

The changes in blood pressure and respiratory movements are shown in figure 1, and the changes in the concentration of the carbon dioxide in the blood plasma are given in table 2.

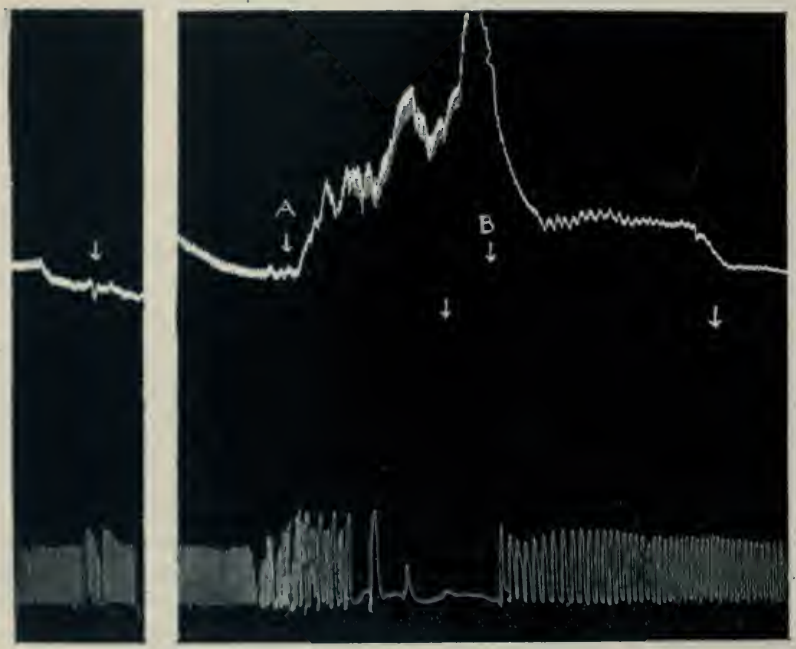

Fig. 1. Upper tracing shows blood pressure taken from one carotid artery. The lower tracing shows costal respiratory movements. The figures below show the concentration of carbon dioxide in the plasma in 10 cc. samples of the blood drawn from the femoral artery at the time indicated by the arrows. The small section at the left of the figure is a control. The concentration of carbon dioxide in the blood was 44.7. Both vertebral arteries had been ligated. At *A* the remaining carotid artery was clamped. The blood pressure rose sharply and the respiratory movements increased. The clamp was removed from the artery at *B*. The blood pressure fell rapidly but remained above normal for a time. A further fall occurred on drawing the last sample of blood. Nine previous samples of blood had been drawn, and the combined effect of hemorrhage and restriction of the circulation to the medulla led to a slowing of respiration after the first marked increase. Respiratory movements were resumed as soon as the circulation to the medulla was restored.

The central respiratory mechanism is sensitive to changes in the volume of blood, since changes in the concentration of the carbon dioxide may appear before the volume of blood withdrawn has begun to affect the systemic blood pressure in any marked degree.

The explanation of the results apparently depends upon two conceptions. First of all, it appears that conditions in the central mechanism itself determine respiratory activity. For it does not appear possible to explain the variations in rate and amplitude of the respiratory movements simply on the basis of the concentration of carbon dioxide in the arterial blood plasma. If the respiratory activity were dependent upon mere concentration of the carbon dioxide in the blood, then one would expect a decrease in rate or amplitude of the movements when the concentration of the carbon dioxide falls. But this is not in accordance with the facts, for the decreased concentration of carbon dioxide in the blood is the result of increased pulmonary ventilation. This increased pulmonary ventilation must result from the change in conditions in the cells of the medulla oblongata itself.

The second conclusion relates to the application of the law of mass action to conditions in the central cells of the nervous mechanism for respiration. If the activity of the central cells from which the efferent impulses in respiratory movements arise depends upon the concentration of hydrogen ions or of carbon dioxide in the cells themselves, we have next to inquire how the volume of blood flowing through or past these cells is related to the conditions in the cells themselves. If the volume of blood flowing through the medulla oblongata in unit time is reduced, each cubic centimeter of blood must take up more carbon dioxide than when the volume of blood flowing through the center is normal. But an increase in the amount of carbon dioxide in each cubic centimeter of blood coming from the central mechanism would inevitably mean a rise in the concentration of carbon dioxide in the nerve cells themselves as a direct result of the solubility coefficient. The direct result of the increase in the concentration of carbon dioxide in the nerve cells would be an increased rate or amplitude of respiratory movements. This would lead to a reduction in the carbon dioxide in the blood. The process would tend to continue until the carbon dioxide in the blood coming to the cells of the medulla was reduced to such an extent that the additional carbon dioxide taken up by each cubic centimeter of the blood leaving the respiratory center did not bring the total concentration of carbon dioxide per cubic centimeter above normal. It is only by such an adjustment that the concentration of carbon dioxide in the cells of the respiratory center could be reduced to and kept at the normal.

The prompt return of the concentration of carbon dioxide to normal when the full volume of blood flow to the medulla oblongata is reëstablished argues against any change in the capacity of the plasma to take up carbon dioxide, as has been suggested in cases of cardiac dyspnoea, nor does there seem any probability of the occurrence of sufficient amounts of acid to account for the decrease in the carbon dioxide of the blood. The hypothesis based on the application of the mass law seems a more much probable explanation of the facts observed. The occurrence of a low concentration of carbon dioxide in cardiac dyspnoea would seem to be a case of much the same sort, as one would expect a decreased volume of blood flowing through the medulla oblongata under conditions of such grave involvement of the heart. The change in the deportment of the cells of the respiratory center is to be expected as a result of the change in the volume of blood flowing through the medulla if, as has been suggested in an earlier paper (4), the constancy of various internal conditions of the organism is to be explained on the general basis of the law of mass action as a means of regulating the degree of activity of the different functional systems of the organism. The application of le Chatelier's earlier theorem, particularly in the form in which it was stated by Riedel (5) that "when any system is in a state of physical or chemical equilibrium, a change in one of the factors of equilibrium will cause a reverse change within the system" seems clear. The volume of blood flow, as related to concentration, is one of the factors of equilibrium. The change in the reverse direction occurring within the system would be an increase in the number or intensity of efferent respiratory impulses, tending to restore the factor of concentration of carbon dioxide to its former normal value.

Changes or reactions of this type occurring within the animal organism have often been spoken of as adaptations. Treviranus, it will be recalled, regarded adaptation as one of the most important characteristics of living matter. Despite the tendency in some quarters to decry adaptation at the present day, we may possibly retain the idea in physiology for some time to come. As has been indicated, we are inclined to regard it as a case under le Chatelier's theorem. And since adaptive reactions are common in higher animal forms, we may find some of the best and most striking illustrations of a general principle of physical chemistry in these forms. Furthermore, we may, by the aid of this general principle, arrive at some of the generalizations of which physiology at the present day stands so greatly in need.

Similar considerations apply to the rise of blood pressure during the period of restriction of the circulation through the medulla. A general

rise of blood pressure would tend to force more blood through the remaining blood vessels than would flow through them under ordinary systemic blood pressure. This would, in its turn, tend to remove more completely any excess of carbon dioxide accumulated in the cells or fluids of the medulla oblongata and, what may be quite as important, convey more oxygen to them. The restriction of the volume of blood flowing through the medulla leads to a change in the system in the reverse direction. This change leads to an increase in the volume of blood flowing through the system.

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THE ACTION OF NEUTRAL ISOTONIC SALT SOLUTIONS IN SENSITIZING ARBACIA EGGS TO THE ACTIVATING INFLUENCE OF HYPERTONIC SEA-WATER

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Received for publication May 21, 1921

Some years ago the senior author found that brief exposure (5 to 10 minutes) to pure isotonic solutions of neutral sodium and potassium salts, especially thiocyanate and iodide, induced typical membrane-formation in unfertilized Arbacia eggs;[1] further, that if such eggs, soon after their return to sea-water, received the usual brief after-treatment with hypertonic sea-water, in accordance with Loeb's method of artificial parthenogenesis, a large proportion (frequently 50 per cent or more) developed to free-swimming larvae.[2] Iodides and thiocyanates were by far the most effective of the salts employed; nitrate had relatively little action, while chloride, bromide, chlorate and acetate left the great majority of eggs outwardly unaltered. The addition of a little calcium (1 mol $CaCl_2$ to 20 alkali salt) to the pure salt solution entirely prevented its membrane-forming action,[2] a similar though less pronounced effect was produced by the addition of certain anesthetic compounds (alcohols, urethanes).[3] Since both the calcium salt and the anesthetic are known to antagonize the toxic and permeability-increasing effects of pure alkali salt solutions on these eggs[4]—apparently because of their having the opposite kind of influence on the physical state of the protoplasmic surface-film—a connection of the latter effect with the membrane-forming action is indicated. A temporary increase in the permeability of the egg-surface to water and water-soluble substances appears in fact to be a regular feature of the activation-process, whether normal or artificial.[5] Apparently the formation of a visible fertilization-mem-

[1] R. S. Lillie: This Journal, 1910, xxvi, 106.
[2] R. S. Lillie: This Journal, 1910-11, xxvii, 289; Journ. Morph., 1911, xxii, 695.
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[5] *Cf.* R. S. Lillie: This Journal, 1916, xl, 249; 1917-18, xlv, 406.

brane is a sign that this surface change has proceeded to a certain critical stage. With membrane-formation is associated a definite change in the physiological properties of the egg; this is shown especially in a greatly increased responsiveness to the activating influence of hypertonic sea-water.[6]

The separation of a visible membrane from the egg-surface is, however, to be regarded merely as the index of some underlying process affecting the properties of the egg-protoplasm and not as the essential process itself. It was found that even when no visible effect was produced upon the eggs, their responsiveness to hypertonic sea-water was distinctly increased. This was observed in eggs that had been treated with calcium-containing solutions of iodide and thiocyanate, and also with solutions of these salts containing an anesthetic (alcohol) in concentration sufficient to suppress membrane formation.[7] It appears, therefore, that an increased susceptibility to the hypertonic treatment may result without any visible change in the eggs. Unfertilized Arbacia eggs which have been exposed to these solutions differ from eggs with definite fertilization membranes in showing no change of form or other external signs of activation when returned to sea-water, and no increased tendency to cytolysis; if left in sea-water without further treatment they exhibit no signs of alteration, but continue to live and even after 24 hours or longer respond to sperm-fertilization in an apparently normal manner.

Treatment of unfertilized Arbacia eggs with pure isotonic solutions of sodium chloride and other neutral sodium salts (nitrate, sulphate, citrate) and also—though to a less degree—with calcium-containing solutions of these salts, has been found during the past summer at Woods Hole to produce a similar increase of susceptibility to hypertonic sea-water without external signs of activation. Brief exposure to pure isotonic solutions of NaCl (0.53 to 0.54m) does not induce membrane-formation, although certain characteristic effects, agglutination and the exit of a little pigment, indicate that this solution produces a definite change in the properties of the cell-surface. Both of these effects, however, are absent in NaCl solutions containing $CaCl_2$ (e.g., 95 vols. 0.54m NaCl plus 5 vols. 0.5m $CaCl_2$); eggs treated with this and similar solutions and returned to sea-water appear quite unchanged; and even after 24 hours or longer still exhibit a normal appearance and respond

[6] *Cf.* J. Loeb: Artificial parthenogenesis and fertilization, Chicago, 1913, chapters 8 to 11.

[7] R. S. Lillie: Journ. Morph., 1911, xxii, 705 *seq.* Journ. Exper. Zoöl., 1914, xvi, 596–600.

normally to fertilization. Yet the responsiveness to hypertonic sea-water is profoundly modified by the brief exposure to the salt solution; this is true of both the pure and the calcium-containing solutions, although the effect is somewhat less in the latter case, especially when the Na-Ca ratio is 10 to 1 or greater. Even after remaining for 24 hours in sea-water after the treatment with salt solution, brief exposure (20 to 40 minutes) to hypertonic sea-water (250 cc. sea-water plus 50 cc. 2.5m NaCl) induces cleavage and development in a large proportion of eggs, many of which—usually a minority but in some cases 50 per cent or more of all eggs—develop to a blastula state. As is well known, exposure of normal untreated eggs to hypertonic sea-water for such brief periods has little or no activating effect.

Brief exposure to isotonic salt solutions thus produces a definite alteration in the physiological properties of the egg, independently of any visible surface-change like membrane-formation. This effect may be described as a "sensitization" to hypertonic sea-water. In certain respects it may be compared with the sensitization to chemical stimulation induced in irritable tissues, such as frog's muscle, by brief exposure to isotonic solutions of NaCl and other sodium salts. The essential features of this phenomenon are as follows. When a fresh normal curarized frog's muscle (gastrocnemius) is brought from Ringer's solution into a pure isotonic solution of a neutral sodium salt (NaCl, NaBr, NaI, $NaNO_3$, etc.) and left there for 3 or 4 minutes, it is found to give a much more vigorous contraction when dipped into a stimulating salt solution (e.g., 4 vols. m/8 NaCl plus 1 vol. m/8 KCl) than when it is brought into this solution directly from Ringer's solution. The muscle also shows a more pronounced response to heat, cytolytic agents, hypertonic Ringer's solution, or other agents that normally cause contractions, than it does in the normal unsensitized state. The general responsiveness to chemical stimulation is abnormally increased by the treatment with the pure salt solution; i.e., a sensitization, which is general and not specific, is induced.[8] In an analogous manner the pure isotonic salt solution renders the unfertilized Arbacia egg more responsive than normally to the activating influence of hypertonic sea-water. It is significant that in both the muscle and the unfertilized egg the effect is decreased or prevented by the addition of $CaCl_2$ to the pure NaCl

[8] *Cf.* R. S. Lillie: Proc. Soc. Exper. Biol. Med., New York, 1910, vii, 170; This Journal, 1911, xxviii, 197; *cf.* p. 214. The remarkable contact sensitivity induced in voluntary muscle by Na-salts which are Ca-precipitants (J. Loeb: This Journal, 1901, v, 362) is another example of a non-specific sensitization.

solution. And since the most constant feature in the general antagonistic action of Ca is a prevention of certain definite and characteristic surface changes in the protoplasm, as shown by the increase of permeability or other demonstrable alteration of protoplasmic structure (e.g., breakdown of cilia) produced by the pure solution,[9] it seems probable that the primary effect in both the above types of salt sensitization is also a modification of the normal physical and chemical properties of the cell-surface. This surface-change in some manner increases the reactivity of the cell to stimulating or activating influences.

This parallel between the sensitization to chemical stimulation in muscle-cells and the increase of susceptibility to hypertonic sea-water in egg-cells is, however, not complete. Differences are seen both in the reversibility of the effect and in its degree of suppression by $CaCl_2$. The sensitization of a frog's gastrocnemius by pure m/8 NaCl is entirely prevented by the addition of $CaCl_2$ to the solution (e.g., in the proportion 1 Ca to 20 Na), and the sensitized state induced by the pure salt solution is completely and rapidly removed by return to Ringer's solution.[10] On the other hand, in unfertilized Arbacia eggs a definite though usually less well-marked sensitization occurs in the presence of a large proportion of $CaCl_2$ (e.g., in a mixture of 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$), or even in pure isotonic $CaCl_2$ (0.35m); and eggs which have been sensitized in pure 0.54m NaCl can be shown to retain their increased responsiveness to hypertonic sea-water for 24 hours or even longer after the return to normal sea-water. In other words, the increased reactivity induced by the solution is not lost or reversed soon after the return to the normal balanced medium, but persists, apparently so long as the egg remains alive. After remaining for 24 or even 48 hours in sea-water the sensitized eggs show a much more complete response to brief treatment with hypertonic sea-water than do unsensitized control eggs. Some permanent modification, possibly the formation of some specific substance or the production of some structural change essential to normal development, has been induced in the eggs by the exposure to the salt solution.[11]

[9] One of the most satisfactory and convenient test-objects for showing both of these effects of the pure NaCl solution (increase of permeability and breakdown of cilia) and their prevention by $CaCl_2$ is the pigmented trochophore larva of Arenicola cristata at Woods Hole.

[10] Unpublished observations made in the Biological Laboratory of Clark University.

[11] It is interesting to note that recovery from the effect of pure NaCl solution on plant cells (Laminaria) is incomplete when the exposure is prolonged

The question of whether the same kind of modification may be produced in unfertilized eggs by other agents, such as light, mechanical treatment, electrical influences or heat, remains for further experiment to determine.[12] The experiments about to be described show that any well-marked change in the balance of salts in the medium may have this effect, although its degree varies with the character of the solution. Treatment with pure NaCl solution is more effective than with mixtures of NaCl and $CaCl_2$ or with pure $CaCl_2$, but apparently departure in either direction from a balanced condition may induce hypersensitivity of this kind.

Experimental. The following records (tables 1 to 5) of experiments with pure isotonic solutions of NaCl and $CaCl_2$, and with mixtures of the two salts in varying proportions, illustrate the essential nature of the results. A large number of experiments was performed during the period from July to September, and the conditions were not found to vary widely from those described in the tables. There is considerable variation in the susceptibility of normal unsensitized eggs to hypertonic sea-water; but typical exposures of $1\frac{1}{2}$ to 2 hours to the solution used (250 vols. normal sea-water plus 50 vols. 2.5m NaCl) are required to induce the development of a significant proportion of such eggs to free-swimming blastulae. The proportion of eggs that form blastulae with this treatment alone is small, rarely more than 5 to 10 per cent; but eggs that have previously been treated for 5 to 10 minutes with isotonic NaCl solution always yield after exposure to hypertonic sea-water a greatly increased proportion of blastulae, in favorable cases 50 per cent or more, and the optimum duration of the exposure is decreased to 30 or 40 minutes; even 20 minutes is often highly effective with such sensitized eggs, although as a rule less than the optimum. These statements refer to temperatures of 20° to 22°.

beyond a certain point; and that a state of imperfect recovery, i.e., one of more than normal electrical conductivity, may persist unchanged for some days after return from NaCl solution to sea-water (Osterhout: Journ. Gen. Physiol., 1920, iii, 145). This suggests the possibility that altered permeability due to the salt treatment may be an essential factor in the sensitization of Arbacia eggs.

[12] With starfish eggs butyric acid exerts its activating influence from two to three times more rapidly when dissolved in van't Hoff's solution containing 3 to 4 vols. per cent ethyl alcohol than when disssolved in van't Hoff's solution alone; i.e., the alcohol has the effect of sensitizing the eggs to the action of the acid (R. S. Lillie: Journ. Biol. Chem., 1916, xxiv, 233: *cf.* footnote, p. 246). Whether alcohol affects similarly the action of hypertonic sea-water remains to be determined.

On the whole the action of hypertonic sea-water on NaCl-treated eggs is less effective than on eggs in which membranes have previously been formed by a fatty acid or a membrane-forming salt-solution such as pure isotonic KCNS or NaI. This fact indicates that the preliminary stage of the activation, as it occurs in NaCl solutions, is only partial; presumably in order to be complete it should proceed to a point at which the egg separates a fertilization membrane on return to sea-water, but this stage is seldom if ever reached with these solutions.[13] Isotonic solutions of other sodium salts, especially iodide and thiocyanate, may, however, produce the complete effect, and their essential action upon the egg is apparently the same as that of typical membrane-forming agents like weak solutions of butyric acid.

The procedure was as follows. The unfertilized eggs were stirred in sea-water to form a uniform suspension, equal quantities of which were placed in the series of dishes (finger-bowls) corresponding to the several solutions of the series (A, B, C, etc.). When the eggs had settled the sea-water was removed as far as possible by pipette, and to the remaining mass of eggs (*ca.* 2 cc.) a relatively large volume of the solution used (100 cc.) was added. After the lapse of the definite period of exposure (usually 5 or 10 minutes) as much as possible of the solution was poured off and replaced by sea-water; the latter was then changed one or more times after the eggs had settled. After a definite period in sea-water (usually 15 minutes) the eggs were transferred to hypertonic sea-water (usually 250 vols. sea-water plus 50 vols. 2.5m NaCl); at the same time eggs of the control unsensitized lot, which had remained in sea-water, were also placed in hypertonic sea-water. Eggs were returned from the hypertonic sea-water to normal sea-water at intervals of 20, 30, 40 and sometimes 50 minutes; the unsensitized eggs received in addition longer exposures, up to 2 hours. The effects upon the eggs (membrane-formation, cleavage, proportion developing to a free-swimming stage) were later determined.

[13] In the starfish egg a certain minimal duration of exposure to butyric acid of a given concentration is required to cause the separation of the fertilization-membrane; with exposures briefer than this minimum no visible effect is produced on the eggs; but that there has been a partial activating effect is shown by the fact that a second equally brief exposure, applied some time later, causes the eggs to form membranes. This result shows that the condition produced in the egg protoplasm by the acid (new compound formed, or structural change, etc.) may persist unchanged for at least a considerable time without external evidence of its existence (for an instance of this effect *cf.* table 17, p. 292 in my paper in Biol. Bull., 1915, xxviii, 260).

Except in the case of pure 0.54 NaCl no visible external change is produced in the eggs by exposure to the above salt solutions. In the pure isotonic NaCl solution, in addition to some increase of permeability, shown in a slight loss of pigment, there is always a tendency for the eggs to cohere in small clumps or agglutinate loosely; this effect is prevented by a very small addition of $CaCl_2$ (1 mol $CaCl_2$ to 400 NaCl was found sufficient); it is absent in the mixed solutions and in the pure isotonic $CaCl_2$, and indicates a definite change in the physical consistency of the cell-surface, probably associated with the toxic or permeability-increasing action of this solution. The sensitization to hypertonic sea-water appears to be independent of this effect. After the return from the salt solution to sea-water the great majority of the eggs show no further change and respond normally to sperm fertilization, even after an interval of many hours. In the experiments of tables 1 to 3 part of the eggs which had been exposed to each of the salt solutions and returned to sea-water were fertilized with sperm next day, about 20 hours later, and in all cases the majority cleaved and developed. A certain proportion of eggs, however, showed evidence of injury, e.g., defective formation of fertilization-membranes and irregular or delayed cleavage; this was especially the case in eggs which had been exposed to the pure NaCl solution; those exposed to the mixed solutions differed only slightly from the normal. It appears, therefore, that apart from exhibiting an increased responsiveness to hypertonic sea-water the eggs are essentially unchanged in their physiological properties and behavior by the exposure to the salt solutions. This is in agreement with what is observed in other cases of sensitization, where the cells show no change in their normal activities except when exposed to certain special conditions (presence of antigen, chemical stimulation, etc.).

Experiments by Miss Baskervill have shown that varying the time of exposure to pure 0.54m NaCl between 5 and 30 minutes has no appreciable effect on the degree of sensitization; but 45 minutes exposure proved unfavorable to further development, probably because of the general toxic action of the unbalanced solution The essential change produced by the salt solution appears to be completed within the first few minutes of exposure. The return to normal sea-water is not a necessary part of the sensitizing procedure. Eggs transferred from the salt solution directly to hypertonic sea-water show the same increased responsiveness as those placed in this medium after an interval in normal sea-water.

TABLE 1

Series of September 2

PREVIOUS TREATMENT FOR 5 MINUTES WITH FOLLOWING SALT SOLUTIONS	APPROXIMATE PERCENTAGE OF EGGS FORMING SWIMMING BLASTULAE WITH AFTER-TREATMENT WITH HYPERTONIC SEA-WATER FOR THE TIMES INDICATED					
	20 min.	30 min.	40 min.	50 min	70 min.	110 min.
A. Pure 0.54m NaCl	1	4–5	50	30–40		
B. 95 vols. 0.54m NaCl plus 5 vols. 0.5m $CaCl_2$	0	1	*ca.* 5	15–20		
C. 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$	0	*ca.* 10	20–25	25–30		
D. None (control with hypertonic sea-water alone)	0	0	1	4–5	*ca.* 5	*ca.* 5

TABLE 2

Series of September 3

PREVIOUS TREATMENT 10 MINUTES WITH FOLLOWING SALT SOLUTIONS	TIMES IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT BLASTULAE				
	20 min.	30 min.	40 min.	60 min.	1 hr. 45 min.
A. 0.54m NaCl	1	2–3	10–15	*ca.* 10	
B. 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$	0	1	1	5–10	
C. 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$	0	1	5–10	1–2	
D. None (control with hypertonic sea-water alone)	0	0	0	1–2	2–3

TABLE 3

Series of September 6

PREVIOUS TREATMENT 10 MINUTES WITH FOLLOWING SALT SOLUTION	TIMES IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT BLASTULAE					
	20 min.	30 min.	40 min.	60 min.	1 hr. 30 min.	2hr.
A. 0.54m NaCl	25–30	5–10	5–10	1		
B. 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$	1	1	*ca.* 1	1		
C. 50 vols. 0.54m NaCl plus 50 vols. 0.35m $CaCl_2$	1	1	4–5	1		
D. Pure 0.35m $CaCl_2$	2–3	3–5	5–10	1		
E. None (control with hypertonic sea-water alone)	0	*ca.* 1	2–3	1–2	1	1

(In this series the effective times of exposure to hypertonic sea-water for unsensitized eggs were less than usual.)

In the case of eggs sensitized in pure 0.54m NaCl the optimum time of exposure to hypertonic sea-water of the above concentration (at *ca.* 20°) is typically between 30 and 40 minutes; in my experience (R.S.L.) 40 minutes exposure has usually given somewhat less favorable results than 30 minutes, and at times 20 minutes has proved the most favorable. In general eggs treated with the calcium-containing solutions require for the best results somewhat longer exposures than those treated with the pure NaCl; while normal unsensitized eggs require much longer exposures ((1½ to 2 hours) and a much smaller proportion develop to larval stages.

TABLE 4

Series of September 9

Unfertilized Arbacia eggs were exposed for 10 minutes to the pure 0.54m NaCl solution. From this solution a part was transferred directly to hypertonic sea-water; the remainder to normal sea-water, and from this, after the intervals given, portions were transferred to hypertonic sea-water. The exposures to hypertonic sea-water were 20, 30 and 40 minutes.

INTERVAL IN SEA-WATER BEFORE HYPERTONIC TREATMENT	APPROXIMATE PER CENT OF EGGS FORMING BLASTULAE AFTER EXPOSURE TO HYPERTONIC SEA-WATER FOR FOLLOWING TIMES		
	20 min.	30 min.	40 min.
1. None (direct to hypertonic sea-water)	25–30	10–15	*ca.* 5
2. 2 min	15–20	*ca.* 25	*ca.* 10
3. 4 min	10–15	10–15	5–10
4. 8 min	*ca.* 15	10–15	*ca.* 5
5. 12 min	10–15	10–15	*ca.* 10
6. 18 min	*ca.* 15	10–15	*ca.* 5

Tables 4 and 5 summarize the results of experiments in which the eggs, after treatment for 10 minutes with pure 0.54m NaCl, were exposed to hypertonic sea-water for 20, 30 and 40 minutes after a variable interval in normal sea-water.

It will be noted that varying the interval between the salt treatment and the exposure to hypertonic sea-water between 0 and 30 minutes has no significant influence on the proportion of eggs developing to the blastula stage. The response of the sensitized eggs to the treatment with hypertonic sea-water shows considerable irregular variation both in the proportion of eggs reaching advanced states and in the optimum times of exposure. On September 9th the best results were obtained after 20 and 30 minutes in hypertonic sea-water, with a well-marked de-

cline at 40 minutes. On September 12th an exposure of 20 minutes had little effect, while 30 and 40 minutes were about equally effective. In a large number of other experiments by Miss Baskervill the optimum exposure was usually found to lie between 30 and 45 minutes. Exposures of 60 minutes and longer were less favorable. With unsensitized eggs the optimum exposures are two or three times longer, as already pointed out.

In a similar series of experiments on September 8th part of the eggs were treated for 10 minutes with pure 0.54m NaCl, and part with a mixture of 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$; then, after intervals in sea-water varying from 0 to 8 minutes, they were exposed to

TABLE 5

Series of September 12 and 13

In both series eggs were exposed to 0.54m NaCl for 10 minutes. Otherwise like the series of September 9, except that the intervals between the successive transfers to sea-water were longer.

INTERVAL IN SEA-WATER	TIME IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT OF BLASTULAE					
	20 min.		30 min.		40 min.	
	September 12	September 13	September 12	September 13	September 12	September 13
1. None (direct to hypertonic)...	1	3–5	*ca.* 5	10–15	*ca.* 5	3–5
2. 5 min.....	1	3–5	*ca.* 5	15–20	*ca.* 5	15–20
3. 10 min.....	1	3–5	4–5	20–25	4–5	*ca.* 20
4. 20 min.....	0	4–5	4–5	15–20	4–5	15–20
5. 30 min.....	0	*ca.* 4–5	3–5	*ca.* 20	4–5	*ca.* 20
6. Control (no salt treatment)....	0	0	0	1	1	1

hypertonic sea-water for the two periods of 20 and 30 minutes. The difference between the pure and the calcium-containing solutions was striking; with the former solution the percentages of blastulae ranged from 3 to 5 with the 20 minutes after-treatment, and from 5 to 20 with the 30 minutes; while with the calcium-containing solution almost no eggs formed blastulae with the 20 minutes exposure and not more than 1 per cent with the 30 minutes. The optimum duration of after-exposure was probably not reached in this case. The anti-sensitizing influence of the calcium was especially well marked in this experiment.

Experiments with pure and calcium-containing solutions of several other sodium salts ($NaNO_3$, $NaSO_4$, Na-citrate) gave results of the same general kind as with NaCl. The sensitizing action was distinct and not

essentially different from that in NaCl. No special peculiarities of action were observed that could be related to the valence or other varying properties of the ions, but the number of experiments was relatively small. The best results were obtained with 5 to 10 minutes in the salt solution, followed by 45 minutes in hypertonic sea-water of the above composition. Exposures of 1 hour to hypertonic sea-water were less favorable.

The experiments of tables 4 and 5 have shown that varying the interval between the two treatments has no significant influence on the proportion of eggs developing to larval stages. Evidently the physiological effect produced by the salt solution is not reversed by the return to sea-water. Even after 24 hours or longer in sea-water the eggs retain apparently unimpaired the increased responsiveness to hypertonic sea-water.

Further experiment has shown that the physiological effects produced by the isotonic salt solution and by the hypertonic sea-water are different in kind, and that the order of the two treatments cannot be reversed. Eggs which have been exposed to hypertonic sea-water for 30 to 40 minutes without previous treatment with salt solution cannot afterwards be induced to develop by treatment with pure isotonic NaCl solution. The only evident effect of the pure salt solution upon eggs thus treated with hypertonic sea-water and returned for an interval to normal sea-water is an increased tendency to cytolysis, and the proportion forming larvae —in any case small with so brief an exposure to hypertonic sea-water— is distinctly decreased. The after-exposure to the salt solution is injurious and gives no increased impulse to development. There is no evidence that it tends to complete the partial activation resulting from the brief exposure to hypertonic sea-water.

The following is a summary of a typical experiment (Sept. 13, 1920). Unfertilized Arbacia eggs were divided into three lots, A, B and C. The eggs of lot A were placed for 10 minutes in 0.54m NaCl and replaced in sea-water. Next day, after 21 hours in sea-water, they were exposed to hypertonic sea-water for 20, 30 and 40 minutes. Those of lot B were first exposed to hypertonic sea-water for 20, 30 and 40 minutes and left in normal sea-water for 21 hours; they were then exposed for 10 minutes to 0.54m NaCl and returned to sea-water. Those of lot C were left untreated in normal sea-water over night and exposed to hypertonic sea-water for 20, 30 and 40 minutes at the same time as lot A. The results were definite and characteristic. Of lot A the percentages of eggs forming blastulae with the three exposures, 20, 30 and 40 minutes, were, respectively, less than 1 per cent, *ca.* 3 per cent, and between 5 and 10

per cent. Of lot B only a few scattered eggs formed blastulae, fewer than in lot C of which less than 1 per cent developed to this stage, even with 40 minutes exposure.

Three other similar series gave the same general result. On September 16th unfertilized eggs which had been left in sea-water for 24 hours after a 10 minutes' exposure to isotonic NaCl solution and were then treated for 40 minutes with hypertonic sea-water, gave 4 to 5 per cent of blastulae; even after 48 hours in sea-water almost the same proportion of blastulae was obtained. The series of September 18th was still more striking; after 24 hours in sea-water the salt-treated eggs gave 10 to 20 per cent of blastulae with exposures of 30 and 40 minutes to hypertonic sea-water; and eggs left for 48 hours in sea-water before the hypertonic treatment again yielded a considerable though smaller proportion of blastulae. On the other hand, when the freshly removed eggs were treated with hypertonic sea-water, left in sea-water for 24 hours, and then exposed to NaCl solution, the only visible effects of the salt treatment were agglutination and increased breakdown. Not the slightest evidence of improved conditions of development was seen in any experiment.[14]

Theoretical. The physiological change induced by the salt solution, involving a greatly increased responsiveness to the activating influence of the hypertonic sea-water, has been described above as a "sensitization;" but such a characterization throws little light on its real nature beyond implying resemblance to the other phenomena of sensitization in living cells. There is little doubt that highly specific forms of sensitization, such as the anaphylactic sensitization of smooth muscle-cells in guinea pigs and other mammals, are referable to the presence in the irritable cell of newly formed specific compounds whose chemical interaction with the antigenic substance gives rise in some manner to strong stimulation.[15] These compounds are the products of a specifically

[14] In the case of Strongylocentrotus Loeb found that eggs subjected first to a somewhat prolonged treatment with hypertonic sea-water (about twice the optimum for eggs with previously separated membranes), and afterwards exposed to the membrane-forming agent (butyric acid), developed in a larger proportion of cases than with the hypertonic treatment alone (J. Loeb: Artificial parthenogenesis and fertilization, p. 110). In this case the order of the two treatments can apparently be reversed without changing the essential character of the results. The time relations are, however, different in the two cases.

[15] The direct condition of stimulation may be a precipitating or other structure-altering effect caused by interaction of the anti-body in the cell-surface with the antigen in the external medium. There are indications that the stimu-

altered metabolism, and it is probable that they are situated largely in the surface-layer of the cell (though they may not be confined to this region); this is indicated by the promptitude with which the sensitized muscle contracts when brought into contact with serum containing the antigen, and also by the phenomena of passive sensitization, which indicate that the circulating antibody is attracted to (e.g., adsorbed) or fixed by the cell surface. In the case of the egg also we may assume that some stable specific substance, which is in some way necessary for development, is formed, or that some definite structural change is produced, as a result of altered metabolism due to the treatment with the salt solution. It is significant that the altered condition is not reversed by the return to the normal medium, as would presumably be the case if (e.g.) simple colloidal changes dependent on altered equilibrium between the salts of the medium and the cell-colloids were concerned. The eggs show no outward evidence of structural change (such as membrane-formation) and they respond normally to sperm-fertilization. Some permanent change in the chemical composition of the internal cell-protoplasm is indicated.

The determining condition of this change is almost certainly some action upon the protoplasmic surface-layer, since it is produced not only by pure NaCl solutions, but also by approximately balanced solutions of NaCl and $CaCl_2$; during the brief period of exposure (5 to 10 minutes) to the isotonic solution there can be little if any penetration of salt into the interior of the egg. Apparently, also, since under these conditions there is no sign of membrane-formation, the separation of a visible fertilization-membrane is an incidental rather than an essential feature of the sensitization-process. The conditions in the Asterias egg, as well as in Arbacia, indicate that a membrane is separated only after the earlier part of the activation-process has reached a certain stage of completion. We may infer that the above salt solutions are less effective than fatty acids or isotonic KCNS or NaI solutions in increasing the responsiveness to the hypertonic treatment because of the incomplete nature of the reaction which they induce. The effect is of the same kind, only partial.

Ultimately the above permanent increase of susceptibility to hypertonic sea-water must be attributed to some definite and specific change

lating reaction and the precipitin reaction are chemically identical, although occurring in different situations and under different conditions. *Cf.* the recent Croonian lecture of H. H. Dale on Anaphylaxis, Proc. Roy. Soc., B., 1920, xci, 126.

in the processes of constructive metabolism occurring in the cell-interior. Apparently this specific metabolic change follows as a more or less direct consequence of altered surface-conditions, just as in many other cells extensive changes of metabolism, e.g., of oxidation-rate, involving the whole active protoplasm, may be initiated by some external process affecting directly only the cell-surface, e.g., the response of a muscle-cell to electrical or mechanical stimulation. The change produced by the salt solution in the physiological properties and reactivity of the egg-system may be the result of altered chemical composition, or of altered structure, or of both combined. It seems probable that some relatively simple initial chemical effect results from the surface-action of the salt solution, and that this leads secondarily to an altered sequence of metabolic processes in the cell-interior. This primary chemical effect might conceivably be a change in the rate or character of the oxidations. According to Loeb and Wasteneys, however, the oxygen consumption in freshly fertilized Arbacia eggs is not changed by isotonic NaCl solution.[16] In the Strongylocentrotus egg, on the other hand, there is a marked increase, according to Warburg.[17] The conditions in the unfertilized Arbacia egg do not appear to have been investigated.

A greater degree of certainty seems to prevail regarding the general nature of the physico-chemical change first produced by the salt solution. Pure isotonic NaCl solution causes an increase of ionic permeability in the plasma-membranes of many and perhaps all cells; to this change must correspond a change of electrical surface polarization. The primary effect of the electric current on living cells is, however (according to Nernst's theory of electrical stimulation[18]), to produce changes of electrical polarization at the semi-permeable surfaces separating the protoplasm from the external medium; evidently this implies—since the current causes stimulation and other changes of activity—that variations in the potential difference across the cell-surface have a controlling influence on the internal metabolism of the cell. It seems probable that the action of salt solutions upon unfertilized eggs belongs in this general

[16] J. Loeb and H. Wasteneys: Biochem. Zeitschr., 1910, xxviii, 340.

[17] O. Warburg: Zeitschr. physiol. Chem., 1910, lxvi, 305. Warburg finds also that various membrane-forming agents increase the oxygen-consumption of unfertilized Strongylocentrotus eggs to a point about equal to that shown by normally fertilized eggs. It thus appears probable that a partial effect, such as that produced in Arbacia eggs by NaCl solutions, would have a similar accelerating influence on oxidations.

[18] Nernst: Arch. f. d. gesammt. Physiol., 1908, cxxii, 275.

class of effects. In any case variations in the salt content of the external medium must influence the conditions of electrical polarization at the cell-surface.

SUMMARY

1. Exposure of unfertilized Arbacia eggs to pure isotonic solutions of NaCl for 5 to 10 minutes (at 20° to 22°) greatly increases the responsiveness to a subsequent brief treatment (20 to 45 minutes) with hypertonic sea-water. Other neutral Na salts (nitrate, sulphate, citrate) have a similar effect.

2. This sensitizing effect of the salt solution may be decreased although not prevented by the addition of $CaCl_2$ to the pure NaCl solution. Apparently it results from any sufficient change in the salt balance. Even pure isotonic $CaCl_2$ solution produces the effect.

3. The sensitized eggs show no other evidence of alteration. They do not form fertilization-membranes and may remain living and fertilizable for 24 hours or more. The sensitized condition is not appreciably reversed during the stay in sea-water, but may persist for so long as 48 hours.

IS CATALASE A MEASURE OF METABOLIC ACTIVITY?

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Received for publication May 24, 1921

When one considers the large number of investigations on catalase, the net result seems exceedingly discouraging, for in all frankness it must be admitted that the functions of this most widely distributed enzyme are still practically unknown. It is therefore to be particularly regretted that this unsolved problem of the significance of the catalase should be further perplexed by a theoretical superstructure of questionable merit. A score of years or more ago Spitzer (13) conceived the idea of a possible connection between the catalase and functional activity. In recent years a similar idea has been greatly elaborated by Burge (4) who offered this as an easy and ready explanation of nearly every problem in the field of biology. It would require too much space to enumerate the various biological phenomena which Burge has brought under the simple hypothesis of the direct relationship between the amount of catalase and metabolic activity.

In his earlier work Burge compared the catalase activity of samples of blood obtained from an animal under different experimental conditions. Where the experiments have been repeated by others, Burge's hypothesis was not corroborated by the new results. Thus, Becht (2), studying the catalase of blood pointed out the important fact that even in a normal animal this is subject to very wide variations (about 1000 per cent!). He demonstrated, furthermore, that the catalytic power of any sample of blood depends upon the number of red cells, a fact which had been recognized already by Löb and Mulzer (8). Becht failed to substantiate the results on the effect of anesthetics which led Burge to formulate the opinion that the lowering of the animal's metabolic activity is accompanied by a parallel lowering in the catalase content of the blood. Reiman and Becker (12) were likewise unable to confirm Burge's results on the effect of anesthesia.

Stehle (14) has gone over several of Burge's experiments which have furnished the arguments in favor of the hypothesis that the amount of catalase is proportional to the oxidative processes in the organism. Stehle, however, failed to corroborate Burge's results in that he either found no change in the catalase content of the blood or only a small increase under the conditions of the experiments. Stehle was also led to believe by the evidence he obtained that the variations in the catalase activity of the blood were due to fluctuations in the number of the red blood cells in the sample.

In recent work Burge has departed from the original technique of experimentation, but is still adhering to his interpretation that catalase is a measure of the vigor and intensity of life processes. Experimenting with potato beetles in different stages of development, he brings forth new evidence in support of his contention showing that as the metabolic activity changes during the life cycle of the animal, the catalase content also increases. Measuring the catalase activity by the number of cubic centimeters of oxygen set free from hydrogen peroxide[1] by 0.5 gram of substance at different stages in the development, from the unfertilized egg to the adult insect, he notes a hundred-fold increase (18 to 1800 cc.). The low rate of oxidation in the unfertilized ovum Burge attributes to its low catalase content. The increase in the oxidative processes in the ovum following fertilization he attributes to an increase in the catalase through augmented production of the enzyme occasioned by the stimulating action of the spermatozoön. From these experiments, in a characteristic manner, Burge makes the deduction that "similarly the increase in the respiratory metabolism or oxidation in youth and its decrease in old age is attributed to an increase in the catalase in the young and to its decrease in the aged." Similar results are also reported with mice of different ages.

It cannot be denied that the catalase content of different parts of an organ, of different functional value, may sometimes reveal quantitative differences. In experiments conducted in conjunction with V. E. Levine (9) on the cortex, medulla and papillary region of kidneys from various animals we found invariably that the catalase content was greatest in the cortex and smallest in the papillary portion. We did not, however, feel justified in ascribing these differences to a metabolic gradient, though we were able to show, by experiments on perfused kidneys, that the variations in catalase are not due to differences in blood content.

[1]Actual amount of hydrogen peroxide not indicated.

Utilizing Edwards' (6) observation on the physiological differences in segments of the Limulus heart, viz., that the contractility of different segments diminishes from the anterior segment backward, the author studied their catalase content but failed to find any parallelism with the physiological observations. Alvarez and Starkweather (1) working with segments from the intestinal tract were able to show that the catalase content follows the functional gradient observed along the intestinal tract. They, therefore, seem to have accepted Burge's hypothesis that physiological function is paralleled by catalase activity.

To put the theory to a direct test I undertook a series of experiments with frogs which were kept at widely different temperatures. It is a definitely established fact that the metabolic activity of poikilothermic animals varies with the surrounding temperature. This was shown by Vernon (15) for frogs as far back as 1894, when he demonstrated that the evolution of carbon dioxide varies with the body temperature of the animals. Cronheim (5), experimenting with fish, found the same relationship between metabolism and surrounding temperature. Thus, at 8°C. carp consumed 600 cc. of oxygen per kilogram and per 24 hours, at 20°C. 2250 cc.; and at 25°C. as much as 2600 cc. In other words, the metabolism increased more than fourfold while the temperature increased about three times. Brunow (3) likewise has shown in his experiments with fresh-water crabs that the carbon dioxide production and the oxygen consumption depend on the temperature of the medium. Within the limits of from 5°C. to 21°C. the metabolism is an exponential function of the temperature, i.e., it increases more rapidly than the temperature. For the range of temperature variations from 5° to 20°C. he found a fourfold increase in the metabolic activity.

Bearing in mind these fundamental facts it seemed to me that they offered an exceptional opportunity for putting Burge's catalase theory to a crucial test. If catalase is a measure of metabolic activity, then evidently the catalase content of frogs kept at widely different temperatures should likewise show large differences.

Before passing on to a description of the experiments and the results obtained, it is necessary to make a digression and discuss some important facts pertaining to the catalase reaction itself which are essential in the interpretation of the results. In a paper on the chemistry of the catalase reaction now in press, the author (10) has shown that this is of the type of a balanced reaction, and that the maximum activity of the catalase is obtained when about 70 per cent of the amount of hydrogen peroxide has undergone decomposition. Furthermore, it has been

shown that an excess of the peroxide causes a dropping off in the amount of oxygen set free by a certain quantity of catalase. However, within the limits of 60 to 100 per cent decomposition the amount of oxygen liberated is a linear function of the quantity of catalase. Theoretically, therefore, it is best to adjust the amount of catalase so that 70 per cent of the hydrogen peroxide will be decomposed. In practice, of course, this is not necessary and it is sufficient to find a suitable quantity of catalase to effect the decomposition of from 60 to 100 per cent of the hydrogen peroxide since this will allow to find the quantity required for 70 per cent decomposition by interpolation. Frequently the calculated amount of catalase has been verified by an experiment but since the determined quantity and the calculated value were always found to check very closely this was not carried out in each experiment.

Another important point to be considered is with regard to the basis which should be assumed in making the comparison of the catalase activity of different preparations. The method generally followed in experiments on catalase has been to determine the amount of oxygen liberated from a certain quantity of hydrogen peroxide by similar amounts of the substance under investigation. The catalase activity is assumed to be proportional to the quantity of oxygen produced. It would take us too far afield to show why this reasoning is theoretically erroneous. Osterhout (11) discussed this question at some length and his article may be consulted in this connection. Suffice it to say here that in attempting a comparison of the activity of different samples of biological material it is necessary to find the respective quantities of material which will effect the same result, i.e., will set free the same number of cubic centimeters of oxygen from a given amount of hydrogen peroxide. In all our experiments the attempt has been made to bring about a 70 per cent decomposition with the same initial quantity of peroxide. The relative activity of the catalase preparations is, therefore, compared on the basis of the respective amounts of the enzyme accomplishing the same work. The catalase activity, of course, varies inversely as the quantities producing the same effect. For instance, if 1 cc. of one preparation and 2 cc. of another give the same quantitative result, the former has exactly twice as much catalase as the latter.

Still another question which must be considered is how long a catalase experiment should be allowed to run. In most experiments on the catalases an arbitrary period has been accepted (usually 10 minutes). Such a procedure is entirely indefensible. Of course, it might be argued that since only comparative values are sought it matters little whether

the reaction is fully or partly completed. This argument might have some force if the tacit assumption that the activity manifested by the catalase is a function of time were true. This, however, is not the case. From the results recorded in a subsequent portion of this paper it will be seen that the reaction is only about 50 per cent complete within 10 minutes. In our experiments, however, the ratio between the catalase and the hydrogen peroxide has been so arranged that the reaction follows the isotherm of a bimolecular equation, and therefore the quantities of oxygen set free in the same periods of time are strictly comparable. When such a condition is not definitely established the comparison on the basis of equal time intervals may have perhaps value from a qualitative but certainly not a quantitative point of view. Although a certain amount of catalase can decompose a definite amount of hydrogen peroxide the final result depends upon two conditions. There must be an optimum hydrogen ion concentration, or at any rate the pH of the reaction mixture should not fall below 6.0. Burge mentions that he uses neutral hydrogen peroxide but does not specify whether this neutrality means a pH of 7.0, or that titration to the zone of neutrality with some indicator like congo red (the indicator which has commonly been used in catalase investigations) is relied upon, in which case the hydrogen peroxide from the point of view of its hydrogen ion concentration is still acid. Again, when the hydrogen peroxide is much in excess of the quantity of catalase with which it is made to react, the amout of oxygen liberated diminishes and the more so the greater the excess of peroxide. In the experiments with the potato beetle material, already referred to, Burge finds that half a gram of unfertilized eggs gives off only one-hundredth the amount of oxygen set free by a half gram of pupae material. Judging by the quantity of oxygen produced Burge must have employed no less than 150 cc. of peroxide ("Dioxogen"). From my own experience with catalases I can say definitely that if the same quantity of peroxide had been used with the egg material—and according to the description of the technique in the papers mentioned this must have been the case—the catalase activity of the latter would be reduced to perhaps one-tenth of its actual strength measured under proper conditions. While this does not imply that there may not be a difference in the catalase strength of the unfertilized beetle eggs and of the pupae, the differences as found by Burge are grossly exaggerated owing to the defect of his experimental procedure.

To come back, however, to the original question as to how long the catalase experiment should be allowed to run. The answer is simple—until the reaction comes to a stop. It is possible, of course, that the reaction is over in 10 minutes provided the *excess* of the catalase is sufficiently large. In that case, however, the excess remains undetermined. In my own experience in standardizing catalase preparations it has not infrequently happened that after repeated trials the quantity just sufficient to bring about complete decomposition of the hydrogen peroxide was perhaps one-tenth of the amount first tested. On the other hand, if the reaction is not complete within the 10 minutes, i.e., if only a fraction of the hydrogen peroxide has undergone decomposition, one is also at a loss inasmuch as the reaction velocities vary directly as the catalase quantities. The tendency will therefore again be for the differences to become exaggerated.

With these preliminary remarks we may now pass to a consideration of the experimental data. Frogs of uniform size and weight were kept for 48 to 72 hours under different temperatures with ample provision for moisture. By placing the receptacle with the frog directly by the ice in a cold box a temperature of 4 to 5°C. was obtained, while the temperature of 30°C. was maintained with a thermostat. The animals were left at these different temperatures for a long enough time to allow perfect reajustment to the changed environmental condition. To forestall any possible criticism that the failure to discover quantitative differences in the catalase content of some particular organ neither proves nor disproves the contention that the metabolic rate is regulated by the amount of catalase present, being due to a shifting of the catalase within the organism, the whole frog was triturated with Berkshire sand in a mortar to a very fine pulp. The ground material was extracted with water saturated with chloroform and the catalase determinations made on aliquot portions of this water extract. In the second series of experiments the frogs were ground without the skins inasmuch as it has been found practically impossible to triturate these. After standing over night in the ice chest the material was centrifuged, the solid residue again extracted with chloroform water, and the total extract made up to a definite volume. The extract was then filtered through a hardened paper. The experiments were performed at 20°C., the volume of the reacting mixture being always 50 cc. The hydrogen ion concentration was directly controlled close to the optimum point (6.9 to 7.3 pH) and the oxygen value of the hydrogen peroxide determined by titration with $KMnO_4$. The results given in this paper have been corrected for temperature and pressure.

Series 1. Frog 1 was exposed to a temperature of 4 to 5°C. for 48 hours. Killed by pithing; total extract was 320 cc. One cubic centimeter of this extract reacting with a quantity of hydrogen peroxide equivalent to 50 cc. of oxygen set free 44.6 cc. In other words, 89.2 per cent of the hydrogen peroxide was decomposed. From this experiment it can be calculated that 0.785 cc. of the extract would effect a 70 per cent decomposition (i.e., would set free 35 cc. of oxygen).

Frog 2. This frog was kept at 30°C. for 48 hours. Prepared in the usual way; the extract was made up to 300 cc. One cubic centimeter of this extract reacting with a similar quantity of hydrogen peroxide produced only 30.8 cc. of oxygen (61.6 per cent of hydrogen peroxide decomposed). From this it may be calculated that 1.136 cc. would be required for a 70 per cent decomposition. The final volumes of the two extracts not being exactly the same, the relative catalase strengths of the two preparations can be regarded only approximately as 1 0.7 (reciprocals of the quantities producing the same amount of work). We may also determine the total catalase content of each extract. Since 0.785 cc. of the cold frog extract can set free 35 cc. of oxygen, the entire extract would liberate 14.27 liters of oxygen. This frog weighed 24 grams, therefore, a gram of the frog substance can set free 595 cc. of oxygen (i.e., it could decompose about 50 cc. of "Dioxogen" hydrogen peroxide). Applying the same calculation to the 30° frog, the total extract could set free 9.1 liters of oxygen ($\frac{300 \times 35}{1.136} = 9100$ cc.). This frog weighed 22 grams; hence, the catalase activity of a gram of frog substance is equal to 414 cc. of oxygen (would decompose approximately 36 cc. of "Dioxogen"). Thus, the frog with the much greater metabolic activity possessed only about 70 per cent of the catalase content of the cold frog. It is possible that at 30° the catalase had actually undergone decomposition within the frog's organism. Such a possibility is suggested by the experiments with frogs kept at 20°C. If this is actually the case, the fact would be most astonishing because a catalase preparation is not destroyed by heat below 60°C. and according to Issajew (7) 40°C. is the optimum. However, it may very well be that the overstimulation of the catabolic processes in the frog by a high temperature involves also the destruction of catalase.

Frog 3. The third frog of this series was kept at 20°C. and has been worked over precisely as the other two frogs. The extract was made up to 300 cc. Using hydrogen peroxide enough to yield 100 cc. of oxygen, it was found that 1.7 cc. of the extract produced 84.8 per cent decompo-

sition. On this basis we may calculate that 1.4 cc. would be required to effect a 70 per cent decomposition. An experiment was performed with 1.35 cc. of the extract with which 66.1 per cent decomposition was brought about. On the basis of this experiment 1.43 cc. would be necessary for a 70 per cent decomposition. It is clear, therefore, that the method of calculation is reliable within 2 per cent. Taking the average of the two calculated amounts, 1.415 cc. of extract can yield 70 cc. of oxygen, or the entire extract would liberate 14.91 liters $\left(\frac{300 \times 70}{1.415}\right)$. The frog weighed 24 grams so that the catalytic capacity of 1 gram of its substance is 621 cc. of oxygen. Considering that the metabolism at 20°C. was probably four times as great as that of the frog kept at 4°C. the difference of less than 5 per cent in the catalase content of the two frogs (621–595) is practically neglible.

Series 2. These experiments were made with two frogs kept for 72 hours at 5° and 20°C. respectively. The animals were prepared in the same way as in the previous series except that the skins were not used. The frogs were each ground up with 8 grams of sand and extracted three times. The final volume of the extract was 300 cc. The experiments were performed at 20°C. using enough hydrogen peroxide to furnish 100 cc. of oxygen. The pH of the mixture was 6.9 to 7.0.

Frog 4. Live weight—18.5 grams; skinned—16.8 grams; temperature 20°C.

Frog 5. Live weight—17.0 grams; skinned—15.0 grams; temperature 5°C.

In each case 2 cc. of the extract produced 81 per cent decomposition of the hydrogen peroxide. The results recorded in the table show a correspondence between the two catalase preparations which could not be surpassed by a duplicate determination with the same sample. In either case the decomposition follows strictly the equation for a bimolecular reaction, the average velocity coefficient in both instances being 0.00075.

From the results presented in the table it can be calculated that frog 4 can set free 12.18 liters of oxygen, or 715 cc. per gram of substance, while frog 5 has a capacity of 12.15 liters, or 810 cc. per gram.

The exposure to widely different temperatures, which it is well known can effect a change in the metabolic rate of from 300 to 400 per cent, obviously had no influence on the catalase content of the frogs. The conclusion is therefore unavoidable that whatever the function of catalase in the organism may be, it is certain that it is not a measure of metabolic activity.

TABLE 1

Cubic centimeter of oxygen evolved by 2 cc. of extract (H_2O_2 = 100 cc. O_2)

TIME IN MINUTES	FROG 4 (AT 20°C.)	FROG 5 (AT 5°C.)
5	27.4	28.2
10	40.3	41.1
15	50.2	51.0
20	58.5	59.1
25	65.6	65.4
30	70.0	69.8
35	73.3	73.3
40	76.1	75.9
45	77.9	77.7
50	79.3	79.3
55	80.3	80.2
60	81.2	81.0

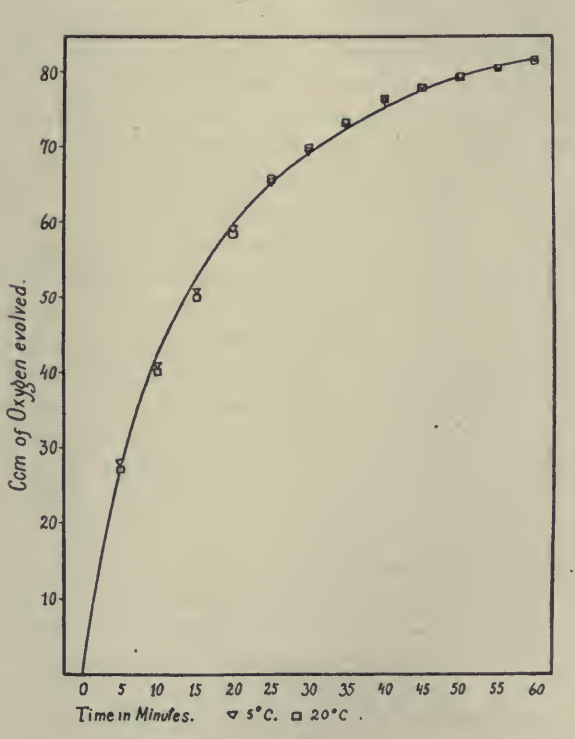

Fig. 1

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Reprinted from The American Journal of Physiology
Vol. 57, No. 1, August, 1921

INFLUENCE OF GLANDS WITH INTERNAL SECRETION ON THE RESPIRATORY EXCHANGE

II. Effect of Suprarenal Insufficiency (by Removal or by Freezing) in Rabbits[1]

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Received for publication May 27, 1921

The fact that animals which survive double suprarenalectomy for a week or more, frequently lose weight rapidly was pointed out by the earliest observers (1), (2), (3), (4). This loss of weight is largely due to the rapid disappearance of fat. Likewise, Porges (5), Schwartz (6) and many others have shown that suprarenalectomy causes a rapid disappearance of glycogen from the liver and a progressive fall in the blood sugar in dogs and rats. Notwithstanding these striking manifestations of suprarenalectomy their possible relation to alterations in the rate of metabolism seems to have attracted very little attention.

Golyakowski (7) in 1899 published a preliminary report on his observations on twelve dogs in which most of the blood supply to the suprarenals was blocked by mass ligation (the exact nature of this operation is not given). He reported that three died within 3 days, one lived 28 days and eight survived longer than 6 weeks. In those animals which survived over 6 weeks, he noted a rise up to 30 per cent in heat production and CO_2 output within the first 10 days, followed by a drop nearly to normal, then a second rise as high as 50 per cent between the 2nd and 4th weeks falling to or even below normal about the 6th or 7th week. Heat production and heat loss were parallel, from which he concluded the heat regulating mechanism was intact. While emphasizing the increase in heat and CO_2 production, he also states there was little or no increase in O_2 consumption above the normal. He realized the discrepancy between the CO_2 and O_2 values, but he

[1] Presented in abstract—Society for Experimental Pathology, Chicago, December 28, 1920.

explained the low O_2 intake by assuming that the extra necessary O_2 was derived from the tissues. On account of this statement together with the absence of any experimental data or descriptions of methods used one cannot attach the importance to his conclusions to which they would otherwise be entitled. We have been unable to find any other references to work on the effect of removal of the suprarenal glands on the respiratory exchange except that reported by Aub (8) and his co-workers. They reported briefly the effects of suprarenalectomy on the respiratory exchange in three cats and found "there was a slight rise in the metabolic rate for some hours, and 48 hours after operation a sharp fall to about 25 per cent below normal. This remained until the animals were sacrificed 5 days after operation." At the same meeting we reported strikingly different observations on the effects of suprarenalectomy on the respiratory exchange in rabbits. It is, therefore, the purpose of this paper to report in some detail the types of modification observed in the respiratory exchange.

Our experience with suprarenalectomized cats has been strikingly similar to that of Aub, except that the deterioration of the animals as measured by the metabolic rate and duration of life was even more rapid. Cats, dogs and guinea pigs usually survive the operation of double suprarenalectomy, whether performed at one or two sittings, only a few days. This fact limits the study of the metabolic changes to the immediate or acute effects of suprarenal insufficiency. White rats withstand double suprarenalectomy too well. The wide variation in duration of life following double suprarenalectomy in rabbits makes it possible easily to obtain metabolic studies on a series of animals surviving the operation from a few days on to indefinite survival. The varying lengths of time which rabbits survive extirpation of the suprarenals is believed to depend upon the presence of varying amounts of accessory cortical tissue, lying for the most part in the neighborhood of the suprarenal glands or along the path of migration of the sex glands. Paradoxical as it may seem the presence of accessory cortical tissue makes it possible to obtain more graded series of incomplete suprarenalectomies than can be obtained by attempting to leave intact varying amounts of the main glands. This is due to the impossibility of preserving unimpaired the blood supply of the unremoved portion. The importance of keeping this in mind becomes even greater if we realize how small a fragment of functionally active suprarenal cortex suffices to maintain the animal in apparently normal health. It was for these reasons that the rabbit was selected as being the most suitable animal for this study.

Methods. The respiratory exchange was determined with the Haldane apparatus, modified as described in a previous paper (9). Rabbits were kept under comparatively uniform conditions. Their diet consisted of alfalfa hay, oats and carrots. They were last fed 15 or 16 hours before the respiratory exchange was determined. The gaseous exchange was determined for a period of 2 hours in each instance. Forty-two rabbits have been used. Control metabolic rates were obtained for a period of several weeks, sometimes before removal of the right suprarenal and sometimes after its removal. No significant change in the metabolic rate or departure from the normal behavior of the animal has been noted following the removal of one suprarenal except in one instance (rabbit 216). In a small group (seventeen) the attempt was made to destroy the function of the cortex of one or both glands by freezing and at the same time to leave the medulla intact. This was done by isolating the gland with as little injury to its blood vessels and nerves as possible and freezing for 30 to 45 seconds with a spray of ethyl chloride. In several instances we succeeded in severely injuring most of the cortex except that portion immediately adjacent or within the medulla, while at the same time preserving most of the medulla with its blood supply intact, as determined later histologically. The method of separating medulla from cortex by freezing, while highly efficient and simple, could not be utilized in effecting a complete separation in rabbits because of the anatomical relationships of cortex and medulla. Likewise, it is not readily applicable to the right suprarenal in this animal because of the relation of the gland to the vena cava. Marked suprarenal insufficiency has been obtained in a number of instances by this method without destroying the medulla as determined by the physiological behavior of the animal and histological examination.

Presentation of data. The following eight protocols have been selected from the 42 experiments of this series as types of the alterations observed in the respiratory exchange. These types may be divided more or less arbitrarily and for convenience of presentation into 3 groups, depending on the completeness of removal and the time of survival, namely:

I. Those that live indefinitely after removal or injury of the suprarenals with no appreciable alteration in the respiratory exchange.

II. Those that show an increase in the respiratory exchange followed by a fall, to, or below normal, whether dying within 2 or 3 weeks or living on indefinitely.

III. Those that show a fall of the metabolic rate beginning within 48 hours after the removal or injury of the glands and continuing to death.

In addition to these groups representing suprarenal injury or removal, it has seemed advisable to add for the sake of comparison one protocol showing the effect of desiccated thyroid feeding in a "normal" rabbit.

GROUP I. *Protocol I.* Rabbit 232; female.

November 26, 1920. Under ether removed right suprarenal completely

December 5, 1920. Wound healed, stitches removed, weight 2810 grams.

December 22, 1920. Began metabolic studies

January 8–11, 1921. Gave total of 0.4 gram potassium iodid by mouth

January 24, 1921. Gave birth to young—destroyed

February 25, 1921. Injected 0.5 gram cholesterol (from gallstones) intraperitoneally

March 22, 1921. Under ether removed left suprarenal completely

March 31, 1921. Wound healed

April 19, 1921. Died this morning, necropsy, body still warm, pregnant near term, 6 embryos; ovaries very large, pale yellow, five distinct corpora lutea; thymus present; thyroids vascular; parathyroids vascular; liver large, pale grayish yellow brown, nutmeg appearance, fatty. Whitish subcapsular deposits probably cholesterol; kidneys enlarged, cortex swollen, grayish and possibly fatty; heart moderate hypertrophy; right and left suprarenal glands absent; no accessories found on careful search; spleen normal in size; solitary gastric ulcer about 1 cm. from pylorus; death probably from acute suprarenal insufficiency developing late in, and in relation to pregnancy with renal and hepatic lesions resembling those of toxaemia of pregnancy.

Protocol II. Rabbit 255, female

March 2, 1921. Began metabolic studies

March 22, 1921. Under ether the right and left suprarenals were exposed through usual incisions for removal and frozen with ethyl chloride spray; the blood vessels were not divided

April 1, 1921. Wounds healed and stitches removed

April 15, 1921. Healthy, strong, killed, immediate necropsy. All viscera appear normal, except suprarenals; right suprarenal easily isolated, upper portion firm, yellow brown, while portion on inferior cava shows soft, vascular, pale yellow, normal-looking cortical mass approximately 3×3 mm.; left suprarenal readily enucleated, upper portion dry, firm, yellow brown with grayish tint, lower portion of gland adjacent renal vein is soft vascular and measures approximately 2×2 mm.; no accessory suprarenals found; both ovaries enlarged, pale yellow and contain several large Graafian follicles; thymus atrophic; thyroid normal in size and color.

GROUP II: *Protocol III.* Rabbit 217, male; adult

September 17, 1920. Started metabolic studies

October 21, 1920. Under ether removed right suprarenal completely

October 28, 1920. Superficial wound infection

November 16, 1920. Under ether removed left suprarenal completely, enlarged

November 18, 1920. Soft stools, dull, eats fairly well

November 28, 1920. Not eating well, getting weaker, wounds healed

TABLE 1

Rabbit 232

DATE	WEIGHT OF RABBIT.	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS*	CALORIES PER KG. PER HOUR	REMARKS
1920									
December 22.	2916	4.295	5.230	0.52	0.46	88	14.83	2.54	November 26. Right suprarenalectomy (complete)
1921									
January 13...	3153	4.675	4.965	0.52	0.40	77	15.64	2.48	
February 9...	2745	4.085	5.095	0.52	0.47	91	14.07	2.56	
February 21..	2764	4.275	4.855	0.54	0.45	83	14.40	2.60	
February 26..	2712	3.770	4.025	0.49	0.38	78	12.55	2.31	
March 1.....	2812	3.730	4.780	0.46	0.43	93	12.98	2.31	
March 4.....	2843	4.505	5.380	0.55	0.48	87	15.39	2.71	
March 8.....	2839	4.120	4.555	0.51	0.41	80	13.92	2.45	
March 16....	2903	4.515	5.350	0.54	0.47	86	15.44	2.66	
March 24....	2800	4.315	4.920	0.54	0.45	83	14.60	2.61	March 22. Left suprarenalectomy (complete)
March 26....	2780	4.375	5.265	0.55	0.48	88	14.93	2.68	
March 30....	2776	3.970	4.400	0.50	0.40	80	13.44	2.42	
April 4.....	2670	3.645	3.945	0.48	0.38	78	12.30	2.30	
April 15.....	2817	4.040	4.200	0.50	0.38	75	13.51	2.40	

* Calculated from CO_2 for all tables.

TABLE 2

Rabbit 255

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
March 2.....	2998	3.895	4.465	0.45	0.38	83	13.25	2.21	
March 10....	3013	3.555	4.070	0.41	0.34	83	12.08	2.00	
March 21....	3026	3.875	4.225	0.45	0.36	79	13.04	2.15	
March 24....	2917	3.585	3.925	0.43	0.34	79	12.11	2.08	March 22. Right and left suprarenals frozen
March 26....	2953	3.880	4.535	0.46	0.39	85	13.21	2.24	
March 30....	2961	3.745	3.980	0.44	0.34	77	12.54	2.12	
April 4.....	2965	3.660	3.945	0.43	0.34	78	12.30	2.07	
April 15.....	2902	3.695	4.035	0.45	0.35	79	12.45	2.14	

December 27, 1920. Slow deterioration to death on this date, immediate necropsy; thyroids not enlarged; parathyroids normal; patchy consolidation of left lung; right lung free; heart not enlarged; both kidneys are small and "spotted." The spots are due to congested depressions in cortex. On section grayish streaks and focal areas are present both in cortex and pyramidal portion; right and left suprarenals absent; no accessories found; thymus large and cellular; lymph glands of mesentery and retroperitoneum enlarged; no visible abdominal fat.

TABLE 3

Rabbit 217

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
September 17.	2424	3.600	3.877	0.52	0.41	78	12.08	2.49	
November 8..	2590	4.180	4.580	0.57	0.45	79	14.13	2.73	October 21. Right suprarenalectomy complete
November 18.	2450	4.385	4.990	0.63	0.52	83	14.81	3.02	November 16. Left suprarenalectomy complete
November 20.	2482	4.330	4.710	0.61	0.48	79	14.54	2.93	
November 22.	2462	4.410	4.775	0.63	0.49	79	14.74	2.99	
November 24.	2582	4.450	5.010	0.60	0.49	82	15.01	2.91	
December 2..	2478	3.730	4.165	0.53	0.43	81	12.60	2.54	
December 8...	2510	4.000	4.715	0.56	0.48	85	13.73	2.73	
December 15.	2522	4.070	4.965	0.56	0.50	90	13.83	2.74	
December 21..	2466	3.370	3.905	0.48	0.40	85	11.37	2.31	

Protocol IV. Rabbit 237

December 7, 1920. Under ether removed right suprarenal completely

December 9, 1920. Began metabolic studies

January 8–11, 1921. Gave total of 0.4 gram potassium iodid

January 25, 1921. Under ether exposed and froze left suprarenal, with ethyl chloride leaving blood vessels intact

January 30, 1921. Fairly active, eats oats, hay and carrot

February 15, 1921. Active, gave birth to one full-term fetus, destroyed

February 18, 1920. Dull, eats very little

March 15, 1921. In good condition, eats moderately of oats, hay and carrot

March 16, 1921. Died about 6 hours after removal from respiratory chamber, necropsy at once; thyroids small, clear, translucent (iodin effect); parathyroids hyperemic; thymus fatty, little lymphoid tissue; lungs clear; heart not dilated; liver normal in size and consistency, congested; spleen congested; pancreas

hyperemic; left suprarenal present as a flaccid, brownish yellow, encapsulated mass, 10 × 6 × 4 mm., no evidence of any normal cortical tissue; microscopically the tissue is nowhere necrotic; a small oval central area in the lower half of the gland around the suprarenal vein approximately 3.5 × 1.5 mm. is composed of normal-looking medulla, taking a normal chrome stain, extremely vascular. The entire medulla appears to be present; toward the periphery of the medulla mass there are islands of large, granular, normal-staining cortical cells. Beyond this the cortical tissue is shrunken, with numerous cholesterol crystal spaces and cholesterol giant cells; the cortical cells are non-granular, nearly stainless or slightly bluish (with H. and E. stain, the cytoplasm of normal cortex cells takes

TABLE 4

Rabbit 237

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
December 9..	2267	3.625	4.035	0.56	0.45	81	12.20	2.69	December 7. Right suprarenalectomy complete
December 14.	2315	3.685	4.790	0.56	0.53	95	12.79	2.78	
1921									
January 13..	2462	3.460	4.800	0.49	0.50	101	12.33	2.50	Figures on basis of 100 R. Q.
January 27..	2550	3.290	3.595	0.45	0.35	79	11.08	2.17	January 25. Left suprarenal frozen
February 2..	2447	3.635	4.400	0.52	0.46	88	12.47	2.55	
February 8..	2389	3.900	4.540	0.57	0.48	84	13.33	2.79	
February 11..	2455	4.145	5.135	0.61	0.53	90	14.29	2.91	
February 15.	2285	3.795	4.280	0.58	0.48	82	12.82	2.81	
February 18.	2252	3.570	4.485	0.55	0.51	91	12.38	2.75	
February 23.	2212	3.390	4.590	0.54	0.53	98	11.96	2.70	
February 28.	2262	3.320	4.105	0.51	0.46	90	11.43	2.53	
March 8.....	2225	3.355	3.550	0.53	0.41	77	11.18	2.51	
March 16....	2486	3.380	3.635	0.48	0.37	78	11.33	2.28	

eosin); the capillaries are intact and contain blood. This is an excellent example of the preservation of almost if not the entire medulla of the left suprarenal with serious injury of nearly all of the cortex with the preservation of a good blood supply to both parts. Two very small, yellowish white nodules on perirenal fat above and external to kidney; right suprarenal absent; both kidneys show shallow, dark red, pitted areas scattered over cortex; on section there are streaks and rounded areas of greyish lymphoid-like tissue in both the cortical and pyramidal portions; ovaries slightly enlarged, follicles present; abundant fat in all depositories; stomach contains fair amount of food, no ulcers present. Death related to period in metabolism chamber.

Protocol V. Rabbit 206; male; adult

April 22, 1920. Weight 2350 grams. Under ether removed right suprarenal completely

April 29, 1920. Wounds healed—weight 2270 grams

June 7, 1920. Began metabolic studies

October 11, 1920. Under ether removed completely left suprarenal, much enlarged, weight 0.461 gram; animal very mangy, coat dull

October 16, 1920. Eats heartily of oats, hay and carrot, soft stools, mange disappearing

October 20, 1920. Mange nearly gone, coat getting glossy, very active, restless, but fatigues easily, wound healed

October 25, 1920. Eats heartily, drinks more water, periods of fatigue and dullness increasing in duration, although restless and active between, losing weight

TABLE 5

Rabbit 206

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
June 7.....	2390	3.570	3.740	0.52	0.40	76	11.90	2.49	April 22. Right suprarenalectomy complete
June 19.....	2400	3.610	4.170	0.53	0.44	84	12.26	2.55	
September 11.	2743	3.365	3.820	0.43	0.35	83	11.33	2.07	
October 12...	2306	3.245	3.410	0.49	0.38	76	10.85	2.35	October 11. Left suprarenalectomy complete
October 14...	2480	4.365	5.625	0.62	0.60	93	15.27	3.08	
October 19...	2405	4.350	4.837	0.63	0.51	80	14.78	3.07	
October 22...	2275	4.635	4.845	0.71	0.54	76	15.42	3.39	
October 25...	2280	4.344	4.827	0.67	0.54	81	14.60	3.20	

October 28, 1920. Died this morning, immediate necropsy, weight 2197 grams; thyroids not enlarged; parathyroids hyperemic; thymus present and cellular; lungs congested, no consolidation; right heart dilated; spleen normal; liver dark brown red and much reduced in size; pancreas hyperemic; right and left suprarenals absent, wound healing complete; no accessories found; stomach small, contracted, three small ulcers in pyloric portion; intestines nearly empty, contracted; testes small, flaccid; no visible fat in the usual depositories; mesenteric and regional lymph glands prominent; typical suprarenal death.

Protocol VI. Rabbit 251; female; adult

February 15, 1921. Began metabolic studies, coat dull, slight mange and alopecia about eyes

March 14, 1921. Under ether exposed right and left suprarenals, through usual incisions and froze both glands with ethyl chloride; blood vessels left intact.

March 23, 1921. Both wounds completely healed, rapid growth of hair over shaved areas; eats heartily of oats and hay and carrot, is gaining weight rapidly, coat becoming glossy, mange about eyes disappearing

TABLE 6

Rabbit 251

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
February 15..	2057	3.250	4.215	0.55	0.52	94	11.35	2.76	
February 24..	2110	3.420	4.075	0.57	0.49	87	11.65	2.76	
March 4.....	2157	2.830	4.040	0.46	0.48	104	10.38	2.41	Figured on basis of 100 R. Q.
March 16....	2105	3.540	4.365	0.59	0.53	89	12.27	2.91	March 14. Right and left suprarenals frozen
March 18....	2283	4.190	6.050	0.64	0.67	105	15.54	3.40	Figured on basis of 100 R. Q.
March 21....	2435	4.300	6.180	0.62	0.65	104	15.88	3.26	Figured on basis of 100 R. Q.
March 23....	2406	4.430	5.310	0.64	0.56	87	15.19	3.16	
March 25....	2556	4.650	6.425	0.64	0.64	100	16.51	3.23	
March 28....	2470	4.405	5.585	0.62	0.58	92	15.29	3.10	
March 31....	2525	4.145	5.115	0.57	0.52	90	14.25	2.82	
April 2.....	2599	4.735	6.385	0.63	0.63	98	16.66	3.21	
April 4.....	2580	4.220	5.090	0.57	0.50	88	14.43	2.80	
April 7.....	2613	4.150	5.140	0.56	0.50	90	14.31	2.74	
April 11.....	2627	3.735	4.745	0.50	0.46	92	12.99	2.47	
April 15.....	2555	3.790	4.070	0.52	0.41	78	12.69	2.51	
April 19.....	2511	2.975	3.515	0.41	0.36	86	10.14	2.02	
April 21.....	2507	3.175	3.620	0.44	0.37	82	10.85	2.16	
April 26.....	2497	3.160	3.570	0.44	0.36	82	10.70	2.14	
May 3.....	2613	3.585	4.445	0.48	0.43	90	12.38	2.37	
May 9.....	2491	3.420	3.885	0.48	0.40	82	11.64	2.34	
May 16.....	2533	3.405	3.990	0.47	0.40	85	11.62	2.29	
May 19.....	2507	3.150	3.670	0.44	0.37	85	10.69	2.13	

April 15, 1921. Clean, sleek, active, well-nourished, eats entire ration

May 1, 1921. Appetite during last 2 weeks seems increased

May 15, 1921. Seems in perfect health, consumes food ration rapidly

May 20, 1921. Sacrificed, animal apparently in excellent health, necropsy; thyroids and parathyroids of normal size and appearance; thymus present and cellular; lungs and heart normal; spleen soft, possibly slightly enlarged; liver dark brown red, possibly somewhat reduced in size, flabby and tough; kidneys

small and smooth, uniformly grayish brown in color; right suprarenal involved in slight adhesions, measures 10 × 6 × 3 mm.; about half of the gland appears normal while the remainder shows the effect of freezing; left suprarenal easily isolated, measures 11 × 7 × 3 mm., there are large masses of normal looking cortical tissue at each pole, while the middle portion is shrunken yellowish brown from the freezing; no accessories found; ovaries not enlarged; abundant abdominal fat; stomach filled with food, mucosa normal.

GROUP III: *Protocol VII.* Rabbit 257; male; adult

March 14, 1921. Under ether removed right suprarenal completely, enlarged, weight 0.40 grams

March 18, 1921. Began metabolic studies

March 30, 1921. Wound healed completely

TABLE 7

Rabbit 257

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER kg. PER HOUR	REMARKS
1921									
March 23....	2250	3.460	4.715	0.54	0.53	99	12.21	2.71	March 14. Right suprarenalectomy complete
March 31....	2290	3.720	4.230	0.57	0.47	83	12.55	2.74	
April 11.....	2315	3.505	4 125	0.53	0.45	86	11.90	2.57	
April 21.....	2214	3.660	4.085	0.58	0.47	81	12.36	2.79	
April 30.....	2105	3.100	3.570	0.51	0.43	84	10.49	2.48	April 28. Left suprarenalectomy complete
May 2.....	2045	3.160	3.825	0.54	0.48	88	10.84	2.63	
May 5.....	1965	2.505	2.745	0.45	0.36	80	8.39	2.13	
May 7.....	1908	2.285	2.470	0.42	0.33	79	7.62	2.00	

April 28, 1921. Under ether removed completely left suprarenal, much enlarged, weight 0.72 gram

April 29, 1921. Active, ate entire ration of oats, hay and carrot

May 1, 1921. Ate very little, looks dull, soft stools

May 7, 1921. Losing weight rapidly, dull, gave 6 grams glucose by mouth

May 8, 1921. Died during night, necropsy; thyroids enlarged and congested; parathyroids congested; thymus present and cellular; lungs and heart normal; liver reduced in size, dark red; spleen normal; pancreas congested; right and left suprarenals completely removed; a 1 mm. grayish, translucent flattened body (accessory cortical mass) on inferior cava just below right renal vein; kidneys appear normal; intestinal tract practically empty; no ulcers in stomach; wounds completely healed; no visible fat anywhere; testes appear normal; typical subacute suprarenal death.

Thyroid Feeding Experiment: *Protocol VIII.* Rabbit 250; adult; female

February 15, 1921. Strong vigorous rabbit. Began metabolic studies

March 4, 1921. Began feeding 0.1 gram very active desiccated sheep thyroid, (containing 0.155 per cent iodin) on alternate days. This thyroid was prepared November 6, 1911, and its pharmacological activity has been tested many times since then, and again this spring on tadpoles, 50 mgm. causing complete metamorphosis in 10 days

March 13, 1921. Looks somewhat dull, fur more erect

March 16, 1921. Gave last of six 0.1 gram doses desiccated thyroid

April 15, 1921. Normal appearance, active

May 15, 1921. Active, healthy, eats all of ration

TABLE 8

Rabbit 250

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
February 15..	2483	3.940	5.595	0.55	0.57	103	14.37	2.89	Figured on basis of 100 R. Q.
February 23..	2425	3.665	4.195	0.53	0.44	83	12.45	2.57	
March 2.....	2455	3.715	4.475	0.53	0.46	88	12.69	2.58	
March 5.....	2413	4.135	4.595	0.60	0.48	81	13.90	2.86	March 4. 0.1 gram thyroid, alternate days
March 7.....	2419	4.020	4.800	0.58	0.51	87	13.73	2.84	
March 9.....	2477	4.400	5.165	0.62	0.53	85	15.04	3.04	
March 11....	2553	4.200	5.650	0.58	0.56	98	14.74	2.89	
March 13....	2490	4.080	5.250	0.57	0.54	93	14.25	2.86	March 14. Last dose thyroid
March 15....	2586	4.815	6.200	0.65	0.61	94	16.70	3.23	
March 17....	2605	4.030	4.755	0.54	0.46	86	13.72	2.63	
March 19....	2665	4.070	4.850	0.53	0.46	87	13.87	2.60	
March 25....	2575	3.695	4.665	0.50	0.46	91	12.88	2.50	
April 7.....	2731	3.630	4.300	0.46	0.40	86	12.41	2.27	

Group I. This group includes those animals thats urvived indefinitely the operation of double removal or injury of the suprarenal glands and whose respiratory exchanges varied less than 10 per cent from their normal average basal metabolism. Fourteen, or 33 per cent, of the rabbits fall in this group. All of these animals with one exception (rabbit 232) that have died or been killed have shown considerable active cortical tissue, either as accessory suprarenals or as unremoved fragments of the main glands or as cortical tissue which had been unin-

jured in the process of freezing. In view of the previous work of other observers and of our previous experience, it is reasonable to suppose that this animal had accessory cortical tissue somewhere to meet its ordinary demands. The failure to find accessory cortical tissue is of little significance since a complete examination would require microscopic examination of serial sections of the entire area within which cortical tissue might occur.

Protocols I and II (rabbits 232 and 255) have been selected as types. In rabbit 232 both suprarenals were removed at two operations, while in rabbit 255 both suprarenals were frozen at a single operation. In rabbit 232 the right suprarenal was removed 26 days before metabolic studies were begun. A series of nine observations extending over the next 3 months was obtained before, and five observations after removal of the second suprarenal. Both glands were completely removed. The respiratory exchange and the body weight remained relatively constant throughout the $4\frac{1}{2}$ months under observation and death occurred near the end of pregnancy associated with lesions of the kidney and liver closely resembling those of the toxemia of pregnancy. In several instances where pregnancy has supervened in animals surviving double suprarenalectomy in apparent health, death has occurred near the end of pregnancy with the lesions above mentioned.

In rabbit 255, three control observations were obtained over a period of 20 days before freezing and five observations over a period of 24 days after freezing both glands. The respiratory exchange and the body weight remained strikingly constant throughout the period of observation. The animal was apparently in excellent health when killed to terminate the experiment. At necropsy while the greater portion of each suprarenal body was injured, there were masses, approximately 3×3 mm. in the right and 2×2 mm. in the left, of normal suprarenal gland containing both cortex and medulla.

Group II. This group includes those animals that showed, following the double removal or injury of the suprarenal glands, increases in their metabolic rates, followed by a fall to or below the normal whether dying within 2 or 3 weeks or living on indefinitely. This increase in the metabolic rate was observed in 24 animals (of which 7 had one or both glands frozen), or 57 per cent of the series and varied from 10 per cent to 63 per cent above the control. The average maximum increase for the group was 23 per cent.

Protocols III, IV, V, VI (rabbits 206, 217, 237, 251) have been selected as types to show the variations in the degree and duration of the increase

and that these effects may be produced either by removal or injury (freezing) of the suprarenals.

In rabbits 206 and 217 both suprarenals were removed at two operations, while in rabbit 237 the right was removed and the left frozen at two operations and in 251 both were frozen at a single operation. The rise in the metabolic rate usually is manifest on the 2nd or 3rd day while the maximum rise occurred in from 2 days to 3 weeks or more. Sometimes the metabolic rate rose gradually to a peak followed by a gradual fall to or below normal. Not infrequently, however, the rate rose and fell two or three times before finally falling to or below normal.

Rabbit 237 showed a rise of approximately 9 per cent, reaching its maximum on the 17th day after freezing the second suprarenal. Rabbit 217 showed a maximum rise of 12 per cent, reaching its maximum 6 days after removal of the 2nd suprarenal. Rabbit 251 showed a maximum rise of 28 per cent reaching its maximum 4 days after freezing both suprarenals, remained approximately at this level for the next 11 days, then gradually fell to or below normal during the next month. During the period when the heat production was increasing rapidly the respiratory quotients for this rabbit were above 1. The animal was killed to terminate the experiment 67 days after freezing both glands, at which time it appeared normal in all respects.

Rabbit 206 showed a progressive rise reaching its maximum of 42 per cent above its normal on the 11th and 14th days, following removal of the second suprarenal. The animal died on the 17th day without any observations after the 14th day. This rabbit lost weight rapidly although he ate voraciously—an excellent example of rapid tissue as well as food oxidation. The rise in all instances was absolute as indicated by both the total heat production and the CO_2 output.

Certain clinical phenomena have been observed in the animals of this group which are strikingly different from those of other groups. The operation wounds healed more quickly and the hair has grown more rapidly over the shaved areas than those of group 1. The nutrition of the skin improved as shown in certain instances where coats previously dull, dry and mangy became clean and glossy. In many instances the animals showed increased appetite. Soft stools so commonly observed following suprarenalectomy in rabbits were always most pronounced during the period of rising metabolic rate. Animals with increased metabolic rates were more active, more restless, more easily irritated and sometimes became vicious. They likewise became fatigued very

quickly. Evidence of increased sexual activity was also observed. In general the animals showing the greatest rise lost weight in spite of a food intake often larger than their average normal.

Group III includes those animals that survived the operation of double removal or injury of the suprarenals from a few hours to a few days. Only 4 or 9 per cent of the rabbits of the series fall in this group. In the case of cats, dogs and guinea pigs, this group would be much the largest. The metabolic rate shows a fall beginning within 48 hours after suprarenalectomy. In certain experiments a slight rise may precede the fall, in others this is absent. The rapidly fatal outcome and the fall in the respiratory exchange are parallel phenomena and depend, it is believed, upon the completeness of destruction of cortical function. Protocol VII (rabbit 257) showing a fall of 26 per cent in heat production below its normal illustrates the group. The period of survival was somewhat prolonged (9 days) but on this account the progressive fall in the metabolic rate is more definite.

Thyroid feeding. Protocol VIII (rabbit 250) is introduced to show the effect on the respiratory exchange of feeding desiccated thyroid to normal rabbits and for comparsion with the effects of suprarenalectomy. One-tenth gram of an active preparation was given by mouth every other day for 12 days. An irregular rise in the heat production reaching 20 per cent above the control rate on the 11th day resulted. The desiccated thyroid was discontinued after 6 doses and the rate rapidly returned to the normal average. The rise in the heat production following the thyroid feeding was less than the average obtained following suprarenalectomy.

DISCUSSION

It appears established that by removing or crippling (by freezing) the suprarenal mechanism a disturbance in the metabolic rate characterized by an increased heat production may be brought about. This disturbance appears definitely related to the completeness of removal of suprarenal function and the duration of life. If only one gland is removed usually no noteworthy changes follow. Even when both are removed there may be no change in the animal's behavior or in its metabolic rate. In such cases one can almost always demonstrate the presence of accessory masses of suprarenal cortex. In most instances (57 per cent of this series) where both glands were removed or crippled there was a progressive rise in the metabolic rate. Sixty-eight per cent of the rabbits in which both suprarenals were removed and 41

per cent of those in which one or both suprarenals were frozen showed an increased heat production. This rise is highly variable both in degree and in duration—no two rabbits showing the same response, irrespective of age or sex. Rabbits show wide variations in their normal basal metabolism though it is relatively constant for a given rabbit over a long period of time. In our series "normals" have varied between 2.1 and 3.2 calories per kilogram per hour, the mean and average being 2.5 calories. Eighty-five per cent varied within 12 per cent of the average.

The increased metabolic rate following suprarenal injury may continue for 1, 2, 3 or more weeks with or without slight remissions and is succeeded by a gradual fall continuing to death or if the animal survives indefinitely, to or slightly below its control rate.[2] Occasionally rabbits surviving indefinitely show more than one period of increased metabolism before the metabolic rates finally become stable at or below the control level. In animals dying within a week following suprarenal injury a rise may not be detected (if observations are made on alternate days, as was the usual procedure in these experiments) or if present, may be quickly succeeded by a decrease continuing until death.

Infection or febrile reaction of other origin or trauma of operation may be eliminated as a cause for the rise. The trauma of operation was approximately the same throughout this series, whether removal was complete or not. But in those where complete removal of the right suprarenal was impossible because of its location, no change in metabolic rate was observed following removal of the second.

The protocol showing the effect of feeding desiccated thyroid was introduced in order to compare the effects on the metabolic rate produced by thyroid feeding with those produced by suprarenal injury. The rise resulting from thyroid feeding was 20 per cent in this instance, which is much less than has been frequently obtained following suprarenalectomy, although the dose used was large and pharmacologically the preparation was very active as shown by its ability to cause complete metamorphosis of tadpoles within 10 days. The comparatively small rise in heat production caused by thyroid feeding suggests that the rabbit is not highly susceptible or is incapable of reacting to the same extent that other animals do. If either of these suggestions is true, and

[2] Rabbits, though to a lesser extent than cats and dogs, show the effect of training. They are more restless the first two or three times they are placed in the chamber. The higher control rates and the lower metabolic rates after a few weeks or months may be thus partially explained.

if the increased heat production following suprarenalectomy depends upon the thyroid, it is probable that animals more susceptible to thyroid feeding will show greater reactions following injury to the suprarenals than will rabbits.

No conclusions have been arrived at regarding the mechanism of the rise in the metabolic rate following suprarenal injury. We have developed a working hypothesis which is as follows: Supposing the intact thyroid gland to be the major factor in the maintenance of a given metabolic rate and that this function is controlled by some regulatory mechanism, we have argued that possibly this control might be exercised by a restraining influence, a major factor in which was the normal function of the suprarenal glands. If such should be the case, the removal or crippling of this restraining or inhibitory influence would allow the thyroid to increase its activity. The frequency with which thyroid and thymus hypertrophy occur after suprarenalectomy in rabbits is additional evidence of the possibility of increased thyroid activity. One cannot neglect to consider the possibility, indeed the probability, of vastly more extensive disturbances in the functions and interrelationships of other organs than the thyroid. There is other evidence that suggests a more general regulatory or inhibitory function of the suprarenal gland. In suprarenalectomized animals there is increased sexual desire, increased intestinal peristalsis associated with soft stools, and increased mobilization of sugar. Wound healing and the growth of hair over shaved areas may be more rapid than ordinarily. The fur over the whole body often became smooth and more glossy. Such animals are usually more irritable and even become vicious. There is sometimes evidence of increased appetite. We have noticed instances where in association with a rise of the metabolic rate there was an increase in body weight associated with respiratory quotients above 1. All these phenomena might be interpreted as suggesting that the suprarenal glands may normally exercise a regulatory action over many body functions. Renal lesions have been observed in a significant number of rabbits surviving suprarenalectomy from 3 to 5 weeks. They have not been detected in rabbits dying within 10 days. The changes have been briefly described in protocols III and IV. Marshall and Davis (10), working with cats, showed there was a definite increase in the urea and creatinin content of the blood in suprarenalectomized cats, but were unable to detect any renal lesions, though carefully looked for. In rabbits we have also observed distinct increases in the urea and creatinin content of the blood in animals after

the onset of the marked terminal asthenia, although at the height of the increased metabolism these substances usually are within normal limits. The possibility of injury to the renal function and subsequent retention of products of metabolism being factors in the increased heat production has not been eliminated inasmuch as Peabody, Meyer and DuBois (11) have shown that occasionally there is an increase in the basal metabolism in cardio-renal cases with cardiac decompensation.

Another question of primary importance arises, namely, is it the medullary or cortical influence which is primarily concerned in the above mentioned disturbance of metabolism. All the evidence at present available indicates that the cortex is the important and the medulla the unimportant tissue as regards maintenance of life. Only those animals survive suprarenalectomy who have functionally active cortex. Epinephrin does not prolong life after suprarenalectomy, and further, the discharge of epinephrin may be nearly if not completely abolished without apparent injury to the animal. As has already been pointed out, if a portion of the cortex, or if only accessories are present, the rise of the metabolic rate may not occur. Also, if most of the cortex is crippled by freezing, leaving the greater part of the medulla intact, as determined histologically, a marked rise may occur. This evidence, although meager, points to the cortex both as the tissue necessary to life and to its being more intimately related to the increased heat production than the medulla. However, freezing experiments do not exclude the medulla as an important factor in this phenomenon since the nerves going to the glands are also frozen and many investigators have shown that the discharge of epinephrin is dependent upon intact nerves (12).

Finally there are many points of similarity between the symptom complex that results from suprarenal injury as above described in rabbits and exophthalmic goiter in man. This possible relationship will be presented in a separate paper.

CONCLUSIONS

1. Removing or crippling (by freezing) the suprarenal glands in rabbits causes a disturbance in metabolism, usually characterized by increased heat production and CO_2 output.

2. This disturbance appears definitely related to the completeness of removal of the cortical function.

3. The symptom complex including both anatomical and physiological data which results from the destruction of the suprarenal function in rabbits resembles in many essential features the symptom complex of exophthalmic goiter.

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Reprinted from The American Journal of Physiology
Vol. 57, No. 1, August, 1921

STUDIES ON NEUROMUSCULAR TRANSMISSION

I. The Action of Novocaine on Muscle Nuclei[1]

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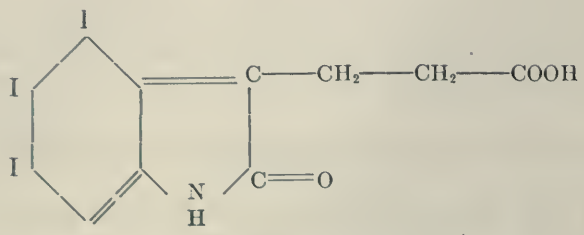
Received for publication May 28, 1921

Introduction. One of the most interesting phases in the growth of biological chemistry is the study of the relation of physiological activity to chemical constitution; that is, the relation between the structure of the molecule and the effect which it produces on the organism. The pioneer in this field of research was Paul Ehrlich, and to his industry and resourcefulness the medical profession owes a debt which it has never fully recognized. His contributions to the study of anemia, immunity and syphilis are well known to professional men, but his theory of physiological activity has never received the attention which it seems to me to deserve, probably because it has not been adequately confirmed by experimental proof.

A drug,[2] said Ehrlich, to be physiologically active, must possess within its molecule two chemical groups; one, a physiologically active group and the other an "anchoring" group. It is the function of the anchoring group to fix the drug to the tissue in which it is to act, and it must, therefore, have a particular affinity for that tissue. The physiologically active group, on the other hand, is, as the name indicates, the portion of the molecule which is responsible for the action of the drug. An interesting example of a substance whose physiological activity may be interpreted in the light of this theory is thyroxin, the active principle of the thyroid gland. The recent investigations of Kendall (19) have shown the structural formula of this substance to be:

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, No. 332, and from the Bermuda Biological Station for Research, No. 128.

[2] The best elucidation of Ehrlich's theory is to be found in his *Studies in Immunity* (8). An estimate of Ehrlich's work has recently appeared in the *Proceedings of the Royal Society*, London, January, 1921.



From what is known of the importance of the iodin atom in the animal body, e.g., in the metamorphosis of tadpoles,[3] and in certain diseases of the thyroid,—it is reasonable to look upon the group which contains the iodin atom as the physiologically active constituent of the molecule; while the remainder of the molecule may be regarded as the anchoring constituent. Moreover, when the anchoring group of thyroxin is altered, the substance loses completely its physiological activity. Thus, in alkaline solution, the imino ring (NH) opens and the substance becomes physiologically inert even though the iodin atoms are still present. This is to be explained by assuming that the alteration of the anchoring group causes it to lose its affinity for the tissues to which it usually attaches itself.

Inasmuch as satisfactory experimental proof for Ehrlich's theory has hitherto been wanting, it appeared desirable to record any experimental evidence which might bear upon the theory.

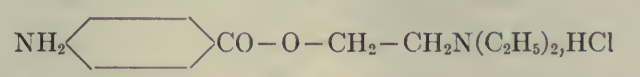
It occurred to the writer that if certain plant alkaloids or anesthetics which are generally supposed to have a special affinity for nervous tissue, could be linked chemically with a staining base, a compound would result which would be a specific nerve stain. In the language of Ehrlich, the alkaloidal constituent of the molecule would be the anchoring group, and the staining constituent, the physiologically active group. Accordingly, the chemistry of the alkaloids and anesthetics was thoroughly inspected in an effort to find one which would be readily amenable to chemical linkage.[4] For this purpose, the most desirable way of linking an organic compound with a staining base is by means of a process known as diazotization. To be diazotized, a substance must possess a benzene nucleus which has attached to it a free amine

[3] A review of the work on the relation of iodin to the metamorphosis of tadpoles will be found in a paper by the writer (10).

[4] The most useful works on the chemistry of alkaloids and anesthetics which have come to my attention are Henry's *Plant Alkaloids* (15) and May's *Chemistry of the Synthetic Drugs* (26).

(NH_2) group. This, when acted upon by nitrous acid, forms the characteristic diazo ($N \equiv N$) group. When placed in the presence of another benzene ring, this diazo constituent becomes linked with it and forms a colored compound. Consequently, if it were possible to find a substance specific for nervous tissue which has a free amine group attached to a benzene nucleus, it could be linked very simply with a staining base.

Diazotization. Of the substances which act upon nervous tissue in the capacity of anesthetics, novocaine appeared to be the most promising for this investigation, since it possesses the necessary prerequisites for diazotization: a free amine group attached to a benzene nucleus. The following formula shows the structure of novocaine:



Having selected novocaine as the anchoring group for the nerve stain, it remained to discover a suitable staining base to link with it. Inasmuch as there are literally thousands of compounds from which to choose, it was almost necessary to resort to a method of trial and error in making the selection. The resulting compound of the staining base and the novocaine must in the first place be soluble in water; it must also retain the physiological activity of novocaine. The diazotization was first performed in succession with *beta*-naphthol, phenol and resorcine, but, owing to the slight solubility of the stains which were produced, they had to be rejected. Neville and Winther's acid was also tried, but with negative results; *alpha*-naphthylamine and *beta*-naphthylamine were little more satisfactory. The next staining base which was tried was a diamine, selected because, as a general rule, diamines are highly soluble in water (urea) and also readily penetrate animal tissues. Such a compound is *meta*-phenylenediamine, and it was found that when linked with novocaine by diazotization, a brown crystalline substance is produced which is highly soluble in water. Table 1 gives a list of the various combinations of anesthetics and staining bases made in the course of the present work. It will be seen that not only novocaine, but also subcutin and propaesin were employed as anchoring constituents in the molecule. The compounds which resulted from the diazotization of the last two anesthetics were in every case only sparingly soluble in water. It remained, therefore, to investigate more extensively the *meta*-phenylenediamine compound of novocaine.

TABLE 1

The diazotization products of novacaine, subcutin and propaesin with various staining bases

ANESTHETIC	STAINING BASE	PROPERTIES	
		Color	Solubility in water
Novocaine	*beta*-Naphthol	Crimson	Insoluble
Novocaine	Phenol	Yellow	Insoluble
Novocaine	Resorcine	Brown	Soluble (1 per cent)
Novocaine	Neville and Winther's acid	Brown	Insoluble
Novocaine	*alpha*-Naphthylamine	Reddish purple	Slightly soluble
Novocaine	*beta*-Naphthylamine	Reddish purple	Slightly soluble
Novocaine	*meta*-Phenylenediamine	Brown	Readily soluble
Subcutin*	*alpha*-Naphthylamine	Purple	Insoluble
Subcutin	*beta*-Naphthylamine	Deep red	Insoluble
Subcutin	*meta*-Phenylenediamine	Bichromate	Insoluble
Propaesin†	*alpha*-Naphthylamine	Pale brown	Insoluble
Propaesin	*beta*-Naphthylamine	Brick red	Insoluble
Propaesin	*meta*-Phenylenediamine	Purple brown	Insoluble

* The formula for subcutin is: $NH_2.C_6H_4.COOC_2H_5$, $HO.C_6H_4.SO_3H$. A description of its synthesis is to be found in May (26, p. 102).

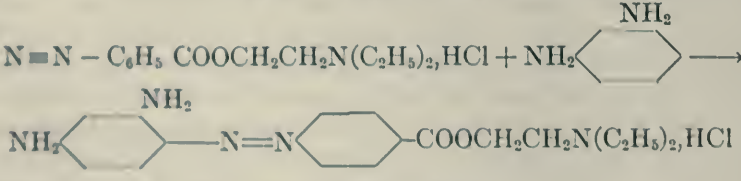
† Propaesin is the propyl ester of *para*-amino-benzoic acid and is represented by the formula: $NH_2C_6H_4COOC_3H_7$.

The chemical reaction involved in the diazotization of novocaine with *meta*-phenylenediamine may be shown as follows:

(1) $NH_2C_6H_4COOCH_2CH_2N(C_2H_5)_2,HCl + HONO \longrightarrow$

$$N{\equiv}N{-}C_6H_5COOCH_2CH_2N(C_2H_5)_2,HCl + H_2O$$

(2) $N{\equiv}N - C_6H_5\ COOCH_2CH_2N(C_2H_5)_2,HCl + NH_2C_6H_4 \longrightarrow$



"Novocaine Brown"

The point on the ring at which *meta*-phenylenediamine will link is, as shown in the last formula, *para* to the first amine group and *meta* to the second. This was concluded from the fact, well-known to the organic chemist, that in diazotization *meta*-phenylenediamine always links in this position (4, pp. 21 and 356).

Physiological action of the diazotized novocaine. Having found that the *meta*-phenylenediamine salt of novocaine (which hereafter will be referred to as "novocaine brown") is freely soluble in water, the problem of its physiological activity immediately presented itself. Are the physiological properties of novocaine brown the same as those of novocaine? A series of experiments was undertaken to test the physiological activity of this dye, and the general conclusion was reached that novocaine brown possesses all the physiological properties of novocaine. The evidence for this came from several sources.

1. It is well known that novocaine, when injected into the body cavity of the Killifish, *Fundulus*, causes the pigment cells (chromatophores) of the scales to expand immediately, giving to the fish its characteristic black coloration. Likewise in the isolated scale novocaine causes the pigment cells to expand. Novocaine brown also produces exactly this expansion, and its reaction-time is practically the same as that of novocaine.

2. When applied directly to the gastrocnemius muscle of the frog, novocaine destroys the reactivity of the muscle to nerve stimuli. To demonstrate this, one makes a preparation of the gastrocnemius muscle with the sciatic nerve attached to it. When the nerve is stimulated with an electric current, the muscle contracts. If, however, the muscle is first painted with a 5 per cent aqueous solution of novocaine, after 10 minutes the muscle will no longer respond when the nerve is stimulated. If novocaine brown is applied in the same way, it produces a like result.

3. It has recently been shown by Göthlin (12) that the plates of cilia in the Ctenophore Beroë when stimulated by a suitable electric current immediately stop beating. However, if the tissue is first treated with atropine or chloral hydrate, the electrical stimulation does not inhibit the ciliary beat; in fact, instances were observed in which there was an actual acceleration of beat in response to the faradic current. Novocaine appears to produce the same effect as the substances which Göthlin employed for, as I have demonstrated, after it has been applied to the mantle of a clam, electrical stimulation of the mantle causes no visible changes in the beating of the cilia. In this regard novocaine brown is equally effective.

From this evidence it seems a reasonable conclusion that novocaine brown retains the properties of novocaine. If, therefore, the assumption made in the beginning of this paper be true,—viz., that novocaine

has an especial affinity for nervous tissue,—novocaine brown should itself possess the properties of a specific nerve stain.

A HISTOLOGICAL STAIN. *1. The prepared stain:* A large number of tissues of frogs and tadpoles in the living state were treated with the prepared stain, and it appeared to have no affinity for nervous tissue. In fact, only the nuclei of the tissues took the stain. Smears made from the spinal cord, sections of the retina and cornea of the eye were treated, and only the nuclei were stained, never the nerve fibers. The possibility therefore suggested itself that novocaine enters all tissues with equal facility but acts only on nervous tissues. In an effort to test this possibility, a process of intra-vital diazotization was resorted to.

2. Intra-vital diazotization: By putting living tissue first into novocaine then into nitrous acid, (washing thoroughly after each reagent), and finally into the staining base, it was found that the stain could be diazotized very successfully directly into the living tissue. By such a process extremely delicate differentiation is possible and in addition the stain is obtained within the tissue in extreme purity. By varying the length of time in which the tissue is allowed to remain in novocaine, and varying also the period of washing, any desired depth of stain may be procured. The first wash removes all of the excess novocaine. If washed a sufficient length of time, moreover, it may be assumed that only that portion of the novocaine which is fixed firmly in the tissue for which it has a particular affinity will remain. In every case it was found that the novocaine had concentrated itself in the nuclei of the cells and never in the nerve fibers. The result of this method is exactly the same as that obtained when the prepared dye is used, save that the elements are more clearly stained.

If cross sections of tadpole material (mounted upon slides) are diazotized directly with novocaine and *meta*-phenylenediamine, an extremely clear histological picture of the material results and, as in the living tissue, the degree of differentiation may be very satisfactorily controlled by altering the time during which the tissue is permitted to remain in novocaine and also by varying the period of washing. The most satisfactory results for such material are to be obtained by leaving the slide in the various solutions for the following periods:

Novocaine (3 per cent)	5 minutes
Wash in water	2 minutes
Nitrous acid	1 minute
Wash in water	2 to 3 seconds
meta-Phenylenediamine[1] (cold)	1 to 30 minutes

[1] One per cent solution in distilled water.

The nitrous acid is formed by adding 2 or 3 drops of weak hydrochloric acid to a 1 per cent solution of sodium nitrite in a Coplin jar. The acid is not added until immediately before using.

From the staining experiments it was at once evident that novocaine does not act upon the nerve fiber. It appeared desirable, therefore, to determine the activity of novocaine physiologically. If novocaine acts only on the nuclei of tissue, it follows that in a tissue devoid of nuclei it should have no physiological effect. Consequently, if a nerve trunk, such as the sciatic, which is composed of nerve fibers without nuclear material, is soaked in novocaine, the power to conduct impulses should not be abolished by the anesthetic.

THE ACTION OF NOVOCAINE UPON NERVE AND MUSCLE. The validity of this assumption regarding the physiological action of novocaine was tested by treating the several constituents of the neuro-muscular mechanism of a frog with novocaine. Sciatic-gastrocnemius preparations were made; the sciatic nerve was then immersed in 5 per cent solution of novocaine, with the result that no visible diminution in its conductivity was to be observed in the course of from half to three-quarters of an hour. This made it evident that novocaine does not inhibit the passage of motor nerve impulses. It had been noted previously that if the gastrocnemius muscle were immersed in novocaine, it could no longer respond to nerve stimulation. However, if the muscle is stimulated directly (by touching the electrodes to the muscle) it contracts readily. This indicates that novocaine, though it prevents the reception by the muscle of nerve stimuli, does not abolish the power of contraction. Inasmuch as the anesthetic does not impede the passage of the nerve impulse, one must conclude that novocaine paralyzes neither the nerve-fiber nor the contractile element of the muscle fiber, but some substance between the two. Since the motor nerves terminate at the periphery of the muscle fibers in structures known as end plates, it would seem that the membrane which separates the end-plate from muscle fiber might be the seat of activity of the novocaine.

It is a curious fact that the Indian-arrow poison, curare, as originally shown by Claude Bernard (1),[6] also acts upon some structure inter-

[6] Pelouze et Bernard (27) were the first to point out that stimulation of the nerves had no effect on a frog which previously had been injected with curare. The explanation of the activity of curare was not published until 1857. Meanwhile, Kölliker (18) had published the details of his experiments with curare. The results of the two investigators were similar. The more recent work on the physiological activity of curare is to be found in the following papers: Langley (22), (23); Garten (11); Cathcart and Clark (5).

mediate between nerve and muscle. Curare, like novocaine, does not affect nerve transmission nor does it destroy the power of muscular response to direct stimulation. Inasmuch as the late war has occasioned a keenly-felt shortage of curare, it seems desirable to emphasize the fact that novocaine offers a very satisfactory substitute for a substance which at the present time is practically unobtainable.

The activity of curare has been explained on the hypothesis that intermediate between a nerve and its muscle there exists a substance, or possibly an actual membrane, which concerns itself with the reception and transmission of nerve stimuli to the muscle proper. The presence of such a substance was long ago urged, and its existence was demonstrated with reasonable satisfaction by Langley (21). In his earlier researches Langley held that an actual membrane existed between the nerve and the muscle, but later he abandoned this idea, and adopted the view that there exists a "receptive substance," which is possibly akin to a chemical side chain,—the side chain, perhaps, of the molecule of which the contractile substance of the muscle fiber itself is composed. The investigations of Lucas and Adrian (25) leave no doubt as to the actual existence of such a receptive substance. They have been able to show by very delicate experiments that the rate of transmission of impulses within this receptive substance is markedly different from the transmission of impulses either in the nerve itself or in the muscle fiber. However, the exact location of the receptive substance has never been definitely ascertained. As a result of the original conception of a membrane separating the nerve fiber from the muscle, it has usually been taken for granted that the place of reception of nerve stimuli is at the surface of the muscle fiber. Indeed, morphologically the nerve fiber certainly ends at the periphery of the muscle. As far as the writer is aware, it has not hitherto been suspected that the receptive substance resides within the muscle fiber itself. Elliott (9, p. 467), however, has noted in his experiments with adrenalin (which he found to act upon the junction between nerve and muscle) that "the irritable substance at the myoneural junction depends for the continuance of its life on the nucleoplasm of the muscle cell, not of the nerve cell." Aside from this observation there appear to be no other experimental results which point to the nucleus as the seat of the receptive substance. It is, however, of interest that Edmunds and Roth (7, p. 45), as a result of their experiments on nicotine-curare antagonism in the muscles of fowls, find that curare acts "upon the muscle substance proper."

It would be decidedly unwise, in a question which is of such great importance, to draw any but well substantiated conclusions, particularly inasmuch as the experiments herein recorded suggest a relationship between the nerve and muscle which differs fundamentally from that which is accepted by most physiologists. The beliefs which are about to be expressed, therefore, are not intended in any way as final, but rather as a working hypothesis, which further experimentation will probably modify.

As has been already noted, morphologically the nerve ends at the surface of the muscle fiber, and, as far as is known, there is no specialized structural continuity between the peripheral membrane and the nuclei within the muscle. Yet novocaine, which destroys the power of the muscle to receive nervous stimuli, acts upon muscle nuclei and apparently acts only there. The evidence for this comes most strongly from the experiment in which a living muscle soaked with novocaine is directly diazotized with a staining base. From the color distribution of the resulting compound, the seat of activity seems very definitely to be the muscle nuclei. Moreover, when the process of diazotization is reversed—that is, by placing the muscle first into the staining base and then into the diazotized novocaine—a diffuse color results, which is not concentrated in any of the tissue elements. It seems certain, *therefore, that novocaine prevents the reception by the muscle of nerve stimuli, because of its action upon the nuclei of the muscle fibers.* With this observation in mind, what can be said of the means of nervous excitation of striated muscle?

It seems probable, in the first place, that the much discussed "receptive substance" of Langley is located within the nuclei of the muscle and, after being acted upon by novocaine, no longer retains the power of exciting the muscle when the nerve is stimulated. Thus, though there may be doubt concerning the existence of a specialized morphological connection between the nerve ending and the nuclear substance of the muscle fiber, it appears that physiologically they are connected most intimately. Moreover, if this view is substantiated, it will demonstrate that the immediate mechanism by which normal muscular contraction is occasioned is the muscle nucleus itself, and that nerve stimuli are received by the muscle nuclei because of the receptive substance residing within them.

The histological conditions which have been demonstrated from time to time tend strongly to support the view that the nerve communicates its impulses to a muscle through the medium of the muscle

nucleus. The axis-cylinder of the motor nerve, it is now agreed, terminates beneath the sarcolemma in an expanded end. Associated with this expanded end are always to be found the "end-plate," or "sole" nuclei (17). In the light of the work on novocaine it is singularly suggestive that there should be such close morphological relation between the motor terminations and the end-plate nuclei. Of much greater significance, however, is the fact that the end-plate nuclei appear to be identical histologically with the nuclei of the muscle fiber proper. Indeed, Huber and DeWitt (17, p. 179) have reached the "conclusion that what has been described as granular sole [end-plate sarcoplasm] may be regarded as an accumulation of sarcoplasma of the muscle. The nuclei of the sole are in structure very similar to the *muscle nuclei, and we have regarded them as such.*" Concerning the motor endings in the striated muscle of the frog Huber and DeWitt (17, p. 183) have again made a most important observation: "In sections giving a surface view of the muscle fibers of the frog, double stained in methylene and alum carmine, it may be seen that the muscle nuclei are more numerous in that portion of the muscle fiber receiving the ramified endings of the motor nerve. Many of these nuclei are found in the thin layer of sarcoplasma in which the axis-cylinder branches terminate. These nuclei are comparable to the so-called sole nuclei, which, it will be remembered, were also regarded as nuclei of the sacroplasma—muscle nuclei."

Other evidence which parallels this is found in the innervation of the heart. "Within the sinus node [the sino-auricular node] . . . . nerve fibers enter . . . . and divide into delicate plexuses from which varicose fibers terminate in simple or complex fashion around the nuclei of the muscle cells" (31, pp. 27–28). Also some of the fibrils of the His-Tawara system "end around the nuclei of the muscle cells."

THE EFFECT OF NOVOCAINE ON THE HEART OF THE CHICK BEFORE AND AFTER INNERVATION. One of the most hotly debated controversies which ever occurred in connection with physiological investigations arose over the question of the origin of the heart-beat. Though of little concern at the present time, the question is still of considerable historical interest. The most important evidence in favor of the myogenic theory of the heart beat came from the observation of His (16), that the heart of the embryonic chick commences beating after 2 days of development, while it does not become innervated until the 6th day. Thus, in the chick there is a very clear instance of heart muscle which beats before nerves reach it.

If, now, novocaine serves only to block the reception by the muscle of the nerve impulse, it should have no effect upon the beating chick heart before it becomes innervated. Embryos of chicks, 3 days old, were very carefully removed from the shell and placed in physiological salt solution, where they were maintained at the incubation temperature; the activity of the heart was observed through a microscope which was so enclosed in a chamber as to keep the desired temperature of 37°C. The rate at which the heart was beating (usually about 50 times a minute) was recorded and then a warm solution of 5 per cent novocaine added directly to the salt solution in the vicinity of the beating heart. There followed, after a few moments, a spasmodic series of contractions, due probably to the alteration in the osmotic pressure occasioned by the novocaine. Within 5 minutes, however, the normal beat became reëstablished. Subsequent additions of novocaine did not seem to affect the beat, and the heart continued its activity in this condition for more than a half-hour.

As was predicted, therefore, novocaine does not affect the contraction rate of the uninnervated chick heart, a fact which gives support to the contention that novocaine destroys only the power to receive nerve stimuli.

On the innervated chick heart, from 6 to 10 days of age, novocaine appears also to be without effect, for when applied directly to a beating heart, which had been removed altogether from the chick embryo, there followed no alteration in the rate of beat. It appears likely, therefore, that novocaine does not affect the heart beat either of embryonic or mature animals; it might well be suspected, however, that after treatment with novocaine, the nervous control of the heart is suspended. To investigate this, the beating heart of a frog was bathed in a strong solution of novocaine (there followed no marked change in the rate of beat)[7] and the vagus nerve exposed for purposes of stimulation. The vagus nerve was first stimulated to test its influence on the heart and having demonstrated satisfactorily that the usual inhibitory effect followed stimulation, the heart was bathed with strong novocaine. After 5 or 6 minutes, the stimulation of the vagus produced no effect upon the pulsation of the heart. Novocaine, therefore, removes the

[7] Roth (29) has made an extensive study of the effect of novocaine on the heart beat of the frog, and finds that when perfused with a strong solution of novocaine, the rate of pulsation is decreased slightly, and that the ventricular contraction is somewhat reduced. When large doses are injected intravenously in higher animals, there follows a depression in the pulse (14).

power of inhibition on the part of the vagus nerve. As in the case of transmission in the sciatic nerve, novocaine does not interfere with the conduction of a nerve impulse in the vagus; consequently, the seat of activity of the novocaine must here also reside in the receptive substance intermediate between the free endings of the vagus and the cardiac tissue. It is of interest that atropine likewise abolishes the inhibitory effect of stimulation of the vagus trunk (21), and that it also is believed to act upon the receptive substance. In the case of cardiac tissue it can very easily be shown by intra-vital diazotization that novocaine has the same action on the muscle nuclei of the heart as in the case of the gastrocnemius, and therefore that the seat of its activity is the same in both cases. It seems reasonable, therefore, to believe that atropine, which acts on the receptive substance, must also act through the nuclei.

The experiments of Göthlin (12) cited above are of especial interest in this connection. When a ciliated tissue is stimulated electrically after treatment with atropine (or novocaine) the cilia continue to beat as usual; when stimulated without such previous treatment, however, their beating stops at once. It has long been known that in certain ciliated tissues there are definite morphological structures connecting the basal bodies of cilia with the nuclei of the cells. Since, therefore, novocaine and atropine remove ciliary inhibition, it is probable that they do so by acting upon the cilia through the nucleus; that the faradic stimulus affects first the nucleus, which in turn inhibits the ciliary beat. If such be the case, it is clear that in ciliated tissue there is a mechanism which apparently duplicates in a surprising way the contractile mechanism found in muscular tissue, where the nucleus appears to excite the cytoplasmic elements of the cell to functional activity.

The effect of novocaine on sensory terminations. Medicinally, novocaine is used as a local anesthetic,[8] and from a practical standpoint, the professional man is more concerned with its effect upon sensory nerve endings than upon motor endings. The experimental results recorded in the present paper deal almost entirely with the effect of novocaine upon motor nerves. In the course of the investigation, however, it seemed advisable to determine whether or not novocaine had a greater affinity for sensory nerve fibers than for motor. About one-quarter of the fibers within the sciatic trunk are sensory. Consequently, when the sciatic nerve is stimulated on one side, the

[8] A discussion of the medicinal uses of novocaine is to be found in Gwathmey's book on anesthesia (13).

leg on the opposite side responds as a result of reflex action. This is due to the well-known fact that when the sensory fibers of the sciatic nerve are stimulated, they transmit the stimulus to the spinal cord, and that, passing through the reflex centers, the stimulus travels down the motor nerve on the opposite side of the body and causes the contraction of the leg muscles. If, before stimulation, the sciatic nerve is treated with novocaine, there is some evidence that the intensity of the reflex is slightly diminished, which possibly indicates that novocaine does interfere with the conduction of such sensory stimuli as are produced by an electric shock.

Though of uncertain effect upon sensory fibers, there is little doubt concerning the action of novocaine upon sensory terminations. When the gastrocnemius muscle on one side of the body is stimulated directly, a prompt reflex is produced on the opposite side; this means that the sensory terminations within the gastrocnemius have received the impulse and have transmitted it through their fibers to the other leg. The same reflex response is secured if the sciatic trunk is exposed above the gastrocnemius and stimulated. If the gastrocnemius is now bathed in novocaine no reflex is produced by direct stimulation of the muscle. The exposed portion of the sciatic trunk, however, will still transmit impulses to the opposite side when stimulated. It is clear, therefore, that in the frog novocaine acts upon the sensory terminations, but has little, if any, effect upon the actual transmission of sensory impulses. It appears to act upon the terminations in such a way as to prevent the reception of impulses, and it is this fact which undoubtedly accounts for its activity in local anesthesia.[9]

In an extensive investigation on the physiological effect of novocaine, Liljestrand und Magnus (24) find that, when injected into the blood stream of a cat, it appears to act upon the proprioceptive (sensory)

[9] It is well known that when a nerve trunk in man is perfused with novocaine, there ensues a complete loss of sensation in all of the tissues which the nerve supplies; however, it does not inhibit materially the passage of such 'sensory' impulses as are produced by inductorium. This result is perhaps the most difficult of all to reconcile with the facts already known concerning the physiological action of novocaine, and it suggests that a normal sensory impulse differs fundamentally in its nature from a motor impulse, or at least from such an impulse as is incited by a faradic current, the true sensory impulses being blocked by novocaine, while motor impulses and those—both sensory and motor —occasioned by an electric shock are quite unaffected by the presence of the anesthetic. Further investigations are now being made in an effort to explain these discordant results.

roots, and in that way destroys the muscle sense. They note also that in large doses it seems to paralyze motor-nerve transmission. The latter result has also been observed by Kubota and Macht (20). It is also interesting to recall that Dixon (6) found that cocaine acted upon sensory nerves more readily than upon motor.

It may very well be, however, that the reception of sensory stimuli by the afferent nerve endings is, as in the case of the efferent endings, effected through the nuclei of the receptive tissues. This would mean that the abolition of sensation by novocaine is due to its action on the nuclei rather than on the sensory nerve endings. The writer, however, does not wish to emphasize this possibility until more substantial evidence has been procured. The fact remains, however, that novocaine when diazotized appears to have no affinity whatsoever for sensory or motor nerve endings.

DISCUSSION

If the observations on the diazotization of novocaine were the only evidence upon which to base the generalization that the receptive substance of Langley is located within the muscle nuclei, the writer would be extremely reluctant to put it forward even as an hypothesis. There are, however, other arguments in favor of the theory which by many will be considered much more convincing than the staining results. This is particularly true of the morphological evidence. The fact that muscle nuclei are crowded into the end-plate is a most suggestive condition and, as far as the writer is aware, no one has hitherto attempted to account for it. In the light of the present theory this concentration of nuclei is readily explained by assuming that they have migrated from the muscle fiber to come more closely into association with the motor terminations. It is extremely interesting, too, that the end-plate as such is found only in muscles of great activity. They are never encountered in smooth muscle of any animal; and in sluggish animals are seldom found even in the voluntary muscles. In the frog the end-plates are best formed in its most active muscles, particularly in the gastrocnemius. Even here, however, the end-plates are rudimentary as compared with those of the higher mammals (see Huber 17. pl. 14; and Boeke, (2, Taf. 17–23); and (3)). An examination of the figures of those who have worked upon the motor terminations makes it evident that the end-plate has increased in complexity, particularly as regards the number of nuclei which it contains, as the muscles have increased in activity.

The idea of a nuclear receptive substance is further supported by the fact that the myo-neural junction is the seat of fatigue. When a nerve is stimulated with a tetanizing current the muscle soon fails further to respond to nerve impulses; when stimulated directly, however, it is found quite capable of contraction. It is generally held that the fatigue brought on in this way is occasioned by an accumulation of the waste products which "congests" the myo-neural junction, and renders it for the time being incapable of receiving nerve impulses. Lactic acid is well known to be one of the chief substances produced by muscular activity, and the lactic acid must be oxidized to enable the muscle to continue its activity. Since it is widely agreed that the nucleus of any cell is a center of oxidation (28), it follows that the muscle nucleus would be the first of the cell elements to be affected by an increase in lactic acid; with continued stimulation the nucleus must literally become overwhelmed with material to be oxidized; as a result it would become fatigued, and would be no longer capable of performing its normal functions.

The writer is well aware of the many difficulties involved regarding muscle nuclei as the junctional tissue, and fails in his purpose if, at any point, he appears to be oblivious to them. In the first place, many will find it difficult to abandon the conception of transmission and contraction as being fundamentally cytoplasmic processes; in response to objections raised on this point, I think it can be said justly that the theory does not in reality require one to abandon such a fundamental conception, but simply to modify it. Transmission is still cytoplasmic, as is also contraction, but the link between the two must be regarded as nuclear. The nucleus merely receives a cytoplasmic impulse and having received it, excites the contractile element of the muscle to activity. Though this explanation may not prove satisfactory to some, it must inevitably be accepted if the theory is to stand.

Perhaps the most vulnerable part of the argument lies in the interpretation placed upon the staining results. Novocaine, it may be urged, might act on elements of a tissue in which it fails to become strongly concentrated; hence the tissues which stain lightly with novocaine brown may be as much affected as those which take a heavy stain. Experimentally this is perhaps the most difficult to meet of all of the objections. However, it seems much more probable that novocaine acts upon that portion of the tissue in which it is most highly concentrated. In fact the entire Ehrlich theory of physiological action is based upon this tacit assumption, and those who object must, I

think, bear the burden of the proof. If it can be shown that novocaine does not act upon the substance for which it has the greatest affinity, all of the deductions drawn from the results of staining immediately fall to the ground.

If further experimentation proves definitely the thesis herein set forth, that the synapse between nerve and muscle is one in which nuclei are concerned, it seems desirable that the function of the nuclei in the spinal synapses should be thoroughly re-investigated with this possibility in view. While the writer does not wish at present to suggest with any conviction that the "synaptic membrane" may be synonymous with nuclear membrane, investigations are now being carried on to ascertain whether or not such is the case. The seat of action of adrenalin is also being investigated.

CONCLUSIONS

The purpose of the present work was to study the nature of the physiological activity of the local anesthetic, novocaine, by application of Ehrlich's theory of drug action. The following are the chief results obtained.

A. Chemical: 1. Novocaine may be linked chemically (diazotized) with a large number of staining bases, and the compounds resulting from such a linkage, when soluble in water, possess the same physiological action as novocaine.

2. The most successful stain, made in this way, was formed by the diazotization of novocaine with *meta*-phenylenediamine. For convenience, this dye has been called "novocaine brown."

3. It may be used histologically as a prepared stain, or it may be diazotized directly into tissue (living or fixed) by dipping the tissue successively into novocaine, nitrous acid, and *meta*-phenylenediamine (washing after treatment with each reagent).

4. Used in either way, novocaine brown stains only the nuclei of tissues, never the nerve fibers, nor the non-nuclear part of the end-plates.

B. Physiological: 1. Applied to sciatic-gastrocnemius preparations, novocaine acts in a way similar to curare, since it does not interfere with nerve conduction nor with muscular contractility, but only prevents the reception by the muscle of nerve impulses. It acts, therefore, on the "receptive substance" of the muscle.

2. By intra-vital diazotization of the novocaine it is shown that the seat of action of the novocaine is in the muscle nuclei.

3. It is, therefore, suggested that the receptive substance of the muscle resides within nuclei of the muscle fibers. This is strongly corroborated by the fact that motor nerves terminate beside the end-plate nuclei—nuclei which have migrated during development from the interior of the muscle fiber.

4. Novocaine does not materially affect the rate of pulsation of chick hearts either before or after innervation.

5. Novocaine nullifies in the clam the "primary inhibition" (Göthlin) of the cilia produced by an electric current, apparently as a result of its action upon the nuclear constituents of the mantle. It seems highly probable, therefore, that the nucleus of a ciliated cell is intimately concerned in the beating of the cilia.

The investigations embodied in the present paper were commenced at the Bermuda Biological Station for Research during the summer of 1920, and were continued in the fall of that year as a research problem in Zoölogy 14,[a] a course given in the first semester by Dr. G. H. Parker. During the second semester of the college year the work was carried on as a research course under Dr. E. L. Mark.

It is with pleasure that I express my indebtedness to Doctor Parker and to Doctor Mark for their encouragement and for the many helpful suggestions which they have given me in the course of the experimental work; also to Dr. J. B. Conant for his advice concerning some of the chemical possibilities involved in the diazotization of novocaine. I wish also to thank Dr. Oliver Kamm, of the Parke-Davis laboratories, for his courtesy in supplying me with many of the drugs used in this investigation.

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TYPES OF OSCILLATIONS IN DIAPHRAGM MUSCLE

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Received for publication May 27, 1921

Mosso (1) in 1886 described oscillations that appear in diaphragm muscle in rabbits and dogs under anesthesia and in normal sleep. Since that time they have been observed by many workers (2). In a series of investigations carried on in this laboratory these oscillations have been observed in urethanized cats, dogs and rabbits and in decerebrate cats and rabbits (3). The purpose of this investigation was to determine the types of forms these oscillations may take.

Methods. The animals used were dogs, cats and rabbits. One series of dogs, cats and rabbits was anesthetised with urethane (2 grams per kilo of body weight) by stomach. The other series consisted of cats and rabbits which were decerebrated under ether. Most of the observations, however, were made under urethane because of its uniform anesthetic action. An animal was fastened back downward with thongs to an animal holder, a cannula inserted into a femoral artery and a mercury manometer attached for recording the blood pressure.

A median incision was made through the abdominal wall and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall. From the S-shaped hook a thread was passed over a pulley to a light writing lever which recorded movements of the diaphragm on a revolving drum simultaneously with the record of the blood pressure.

In the early experiments a simultaneous record was obtained of the movements of the chest wall along with that of the diaphragm muscle but it was found either to move synchronously with the diaphragm or not to move at all. The chest record was omitted in the later experiments. Also attempts were made to record by means of a tambour but this method did not prove sufficiently delicate for the small oscillations.

Continuous observations were made on these animals for periods varying from 6 to 10 hours. Inspirations are represented by down

strokes of the muscle lever and expirations by the up strokes. The temperature of the animal was maintained by means of an electric heater.

Results. In considering the results of this work it should be stated that the oscillatory rhythms appeared most prominently in the diaphragm muscle of vigorous animals, yet they failed to appear in some

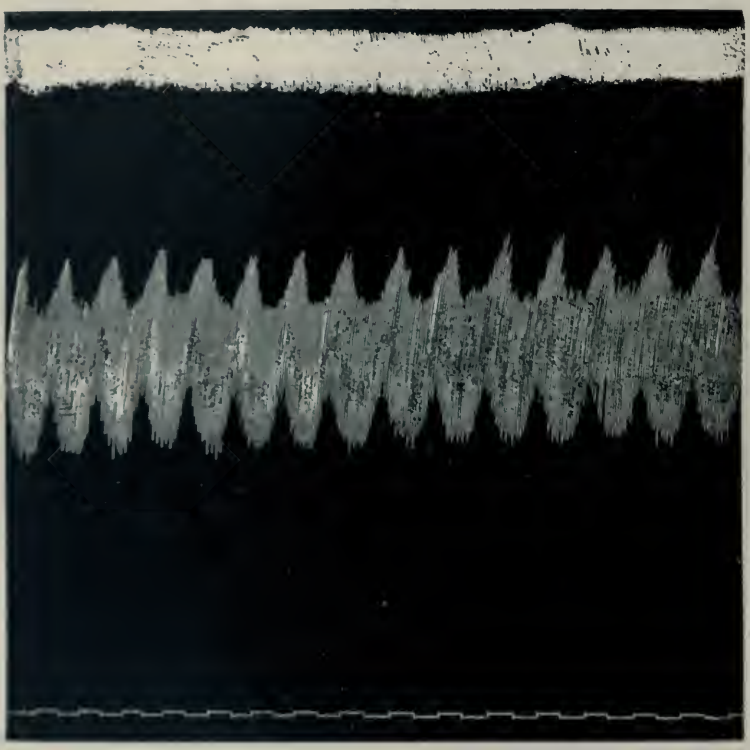


Fig 1. Dog. The upper curve indicates the blood pressure; the middle curve, contractions of the diaphragm muscle; and the lower line, the time at 5 second intervals.

that seemed normal. Another fact of general interest is that these oscillations may appear at any time from within 1 to 6 hours after the observations are begun.

Types of oscillations. Figure 1 shows a type of curve that appeared in a dog about one hour after the experiment had begun. It is seen that these oscillations occur both at the bottom and the top of the curve

and the amplitude is nearly uniform in all phases. Since the down strokes represent inspirations it indicates that the chest cavity is larger and would hold more air when the oscillations appear at the bottom of the curve, and vice versa when they appear at the top.

Figure 2 which was obtained from the diaphragm of a decerebrate cat shows a variation from the type in figure 1. The oscillations are somewhat greater at the bottom of the curve than at the top, the amplitude is not uniform and wide excursions of the lever occur from time to time as a result of gasps.

Fig. 2. Decerebrate cat. Oscillations irregular in amplitude and greater on the down strokes of the lever.

In figure 3 the oscillations take on an entirely different form inasmuch as the rhythms appear only at the top of the curve and indicate a decrease in the tonicity of the diaphragm. These oscillations appeared about 6 hours after the beginning of the observations and continued for 5 hours except for intermissions of 1 to 10 minutes which occurred from time to time until the experiment was terminated. During the course of the experiment the oscillations appeared at fairly regular intervals, then would disappear for a minute or more to reappear singly, in pairs or in trios, and then disappear and reappear later in the same manner. As the experiment proceeded the height of the oscillations became smaller.

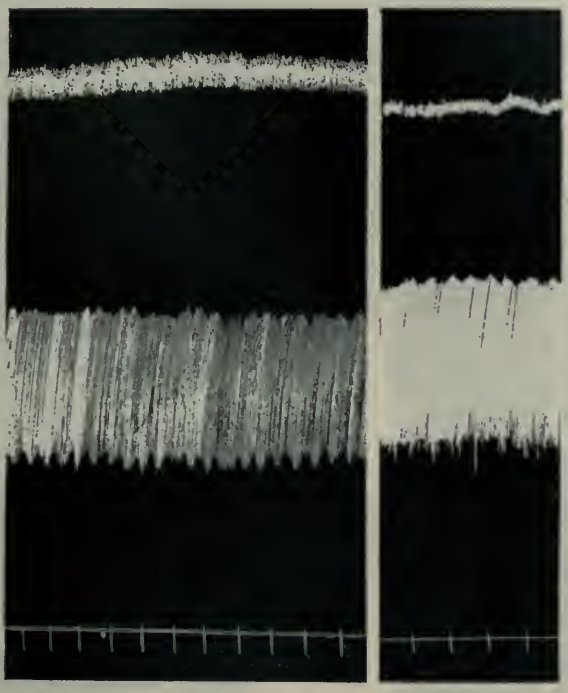
A fourth type of curve is represented in figure 4A where the oscillatory rhythms appear at the bottom of the curve. This is a fairly com-

mon type. In this animal these oscillations appeared and then disappeared and then about 30 minutes later they had entirely changed their form and appeared at the top of the curve instead of the bottom as shown in figure 4B.

Fig. 3. Cat. Oscillations at top of curve. Time, 5 seconds

A fifth type of curve observed in these experiments is represented in figure 5, which shows compound oscillations. It is seen that large oscillatory waves appear in the diaphragm curve about every 2 to 3 minutes. These large rhythms first occurred nearly 5 hours after

the experiment was started. Another significant fact about this curve is that small oscillations are superimposed onto the large ones, especially on the right half of the curve giving compound oscillations.



A B

Fig. 4. Cat. Oscillations at bottom of curve in *A* and at top of curve in *B*. Time, half-minutes.

DISCUSSION OF RESULTS

Relation to blood pressure. On examining these curves it is seen that there is no direct relation between oscillatory waves of the diaphragm and changes in the blood pressure. This has been our uniform experience with dogs and cats. In the rabbit, however, we have observed a direct relation of blood pressure to oscillations in the diaphragm in some of our experiments, similar to those described by Mosso (4).

Source of oscillations. The oscillations in diaphragm muscle are usually considered to be due to influences arising from central origin (5). It is well known that changes in the carbon dioxide content of the blood

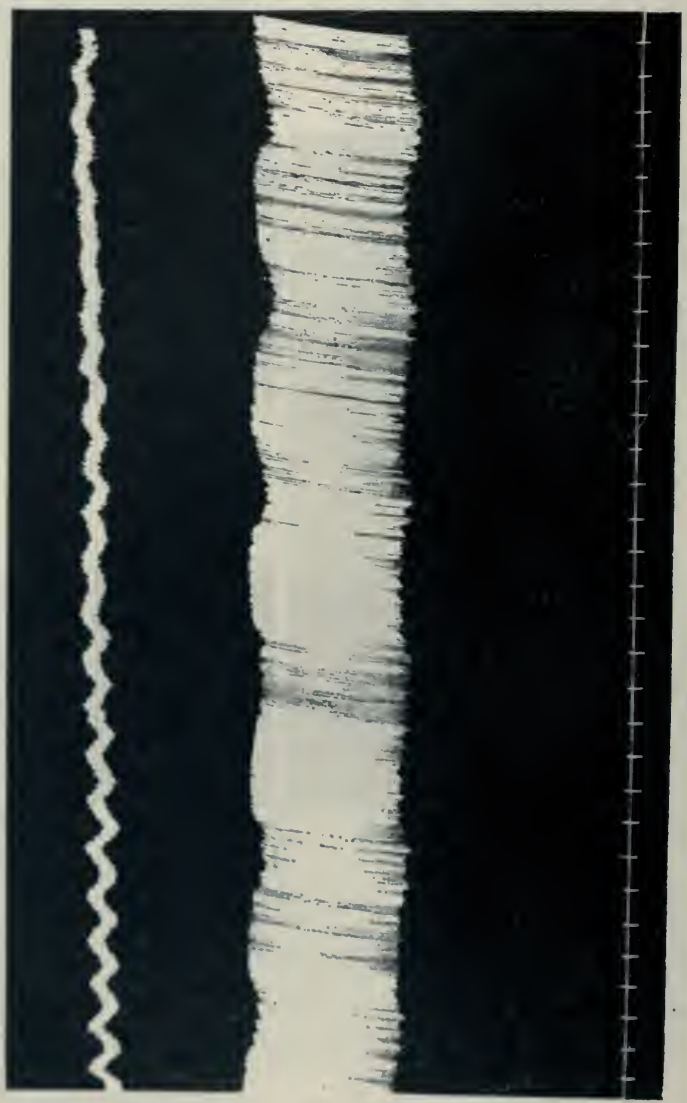


Fig. 5. Cat. Compound oscillations of diaphragm muscle. Time, half-minutes

will influence the depth of respiration. We have tried experimentally to evoke these rhythms by administering CO_2 by way of the lungs, but without success. Mosso observed oscillatory rhythms in the diaphragm and chest muscles of the dog after apnoea, which was produced by artificial respiration (5). Macleod in a recent paper has shown that "periodic breathing" may occur in decerebrate cats when the oxygen supply is reduced and that on administering oxygen the breathing returns to normal (6). In decerebrate cats and rabbits and in urethanized dogs and cats we have had oscillations appear in the diaphragm muscle when the excursions of the writing lever were greatest, indicating increased ventilation, and disappear when they were least, and vice versa. We have also had oscillations appear as in figure 3, when there are wide excursions of the lever and disappear, then later reappear and disappear when the rate of breathing is slower and the amplitude of the curve not more than two-thirds as high. This would seem to indicate that other factors besides oxgyen may influence these rhythms.

Recently we have shown that oscillations may appear in diaphragm muscle *in situ* when the medulla is pithed and the phrenic and vagi nerves are severed and the peripheral end of a phrenic nerve is stimulated rhythmically with uniform break induction shocks (7). Oscillatory rhythms have also been obtained in a similar way on various other muscles (8). Lee, Guenther and Meleney found "rhythmicity" appearing in isolated diaphragm muscle of the cat when stimulated with induction shocks at a uniform rate (9).

The fact that oscillatory rhythms may occur in diaphragm muscle when isolated from the respiratory center and stimulated with break induction shocks through a peripheral end of a phrenic nerve suggests the possibility that the oscillations appearing when the nervous system is intact may be due in part to changes in the irritability of the muscle itself.

We have found no direct indications that the oscillations in diaphragm muscle are due to fatigue. They may appear soon after the observations are begun or several hours later.

SUMMARY

1. Five types of oscillations that may appear in the diaphragm muscle of urethanized and decerebrate animals are presented.

2. These oscillations may take different forms and they may appear, disappear and reappear at any time within 1 to 6 hours after the observations are begun.

3. In general there seems to be no direct relation between the character of the oscillations in diaphragm muscle and changes in the blood pressure.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 57 SEPTEMBER 1, 1921 No. 2

ON THE ARTIFICIAL EXTRAPAUSE OF THE VENTRICLE OF THE FROG'S HEART

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Received for publication March 4, 1921

Dastre (1) and Langendorff (2) were the first to show that sometimes after applying an artificial stimulus to the auricles of the frog's heart, a prolonged ventricular pause arises, which is not initiated by an extrasystole of the ventricle. Engelmann (3) was in position to corroborate this experiment and to elucidate it. He pointed out that the experiment succeeds only when the stimulus is given to the auricles at the commencement of the ventricular systole, after which an extrasystole of the auricles will ensue. After this the excitation wave proceeds to the ventricle and reaches it before the close of the refractory stage, so that no ventricular systole follows. Only after the compensatory pause which succeeds the extrasystole of the auricles do the auricles and the ventricle resume their normal rhythm. This experiment, however, seldom succeeds. It is instanced in figure 1. At *T* the first upward deflection of the signal the auricles were given an induction shock[1] at the commencement of the ventricular systole. After the auricular extrasystole evoked by this shock the excitation reached the ventricle during the refractory stage, so that no systole of this chamber arose.

Not before the end of the compensatory pause of the auricles did an auricular systole arise again, followed by a ventricular systole. I have now been more successful in this experiment, by lengthening the dura-

[1] In all figures the closing of the primary circuit was indicated by a downward deflection of the signal. At the opening of the primary circuit an upward deflection of the signal was effected. In figures 1, 2, 3, 4 and 6 the closing stimuli were shut off and consequently they did not reach the heart.

tion of the refractory stage of the ventricle. Then the excitation wave after the artificial extrasystole of the auricles will with greater certainty reach the ventricle still in the refractory stage. This lengthening of the refractory stage of the ventricle may be effected in different ways. First of all we know ever since Langendorff wrote, that the duration of the postcompensatory systole has increased. I now found that during the postcompensatory systole also the duration of the refractory stage has increased. It may be expected, therefore, that the experiment succeeds better during a postcompensatory systole. This may be seen from figure 1, in which the auricles received a fresh stimulus during the postcompensatory systole at the second upward deflection of the signal, and hereafter followed another extrapause of the ventricle, which was not preceded by a premature ventricular systole.

At *P* the third upward deflection of the signal the auricles were again stimulated at the commencement of a ventricular systole. After the evoked extrasystoles of the auricles the excitation wave reached the ven-

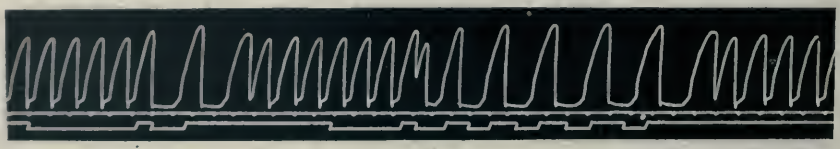

Fig. 1

tricle after the refractory stage, so that a premature ventricular systole ensued. When, however, at the next upward deflection of the signal the stimulus is repeated at the commencement of the postcompensatory systole, the excitation wave after the extra-auricular systole thus evoked, readily reaches the ventricle during the refractory stage. Now an extrapause of the ventricle follows. In this way it is easy to repeat the experiment during every following ventricular systole, which is broadened every time. At last it is even unnecessary to stimulate the auricles at the commencement of the ventricular systole, the last stimulus being given about the middle of the ventricular systole without diminishing the success of the experiment. This, indeed, is easily understood, if we look more carefully at the ventricular systoles of this artificial halved ventricular rhythm. We then observe that after the compensatory pause the postcompensatory systole is broader than the preceding ventricular systoles, and that every succeeding systole surpasses its predecessor in broadness. We see then that the contractility of the ventricular

muscle increases after every lengthened ventricular pause. This restoration of the ventricular muscle in the artificial halved rhythm involves an increase in duration of the refractory stage from systole to systole. This is why ultimately the stimulus can be administered to the auricles later in the ventricular period, without interfering with the success of the experiment. After the last stimulus the ventricle resumes again the normal rhythm.[2] In the second place we can lengthen the refractory stage of the ventricle by poisons, namely digitalis, veratrin, antiarin or barium chloride and, by doing so, ensure success of our experiment. The curves of figure 2 refer to a frog's heart that had been poisoned with barium chloride. At every upward deflection of the signal the auricles receive an opening induction shock at the commencement of a ventricular systole. Every time there appears an extrasystole of the auricles and every time after this the excitation reaches the ventricle during the refractory stage, so that extrapauses of the ventricle orig-

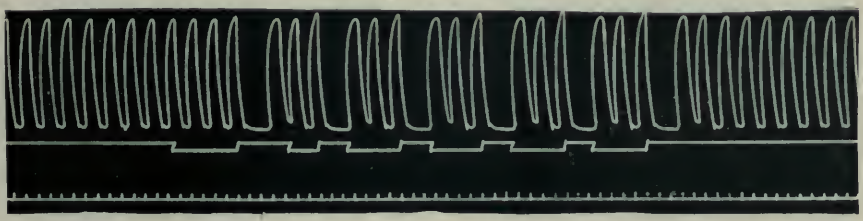

Fig. 2

inate, which are not preceded by premature ventricular systoles.[3] I have now detected that artificial extrapauses of the ventricle may be evoked in the frog's heart in quite another manner. Whereas in the method described above, the prolongation of the refractory stage of the ventricle was the decisive factor, the following method is based on a principle unknown as yet in the physiology of the heart. When we place the stimulating electrode in the auriculo-ventricular groove, we can evoke under certain circumstances (prolonged refractory stage of the ventricle), by the administration of an extrastimulus toward the

[2] After poisoning with veratrin, digitalis, antiarin or barium-chloride, the halved rhythm of the ventricle can persist after one or more extra-pauses of the ventricle, without stimulating the heart any more. This also can occur after bleeding the non-poisoned frog's heart. (See fig. 5.)

[3] In a later stage of this intoxication the ventricle maintains its pulsation in the halved rhythm after such an artificial extrapause.

close of the diastole of the ventricle, an extrapause of the ventricle, which is not preceded by an extrasystole of this chamber.

In our experiments described above we had to give the extra stimulus at the beginning of the systole to obtain the desired result. When the stimulus was given a little later a premature ventricular systole succeeded the extrasystole of the auricles.

It is obvious, then, that when a stimulus at the end of the diastole of the ventricle produces the same effect, it cannot be explained in the same way. We shall therefore illustrate the latter experiment by some curves. In figure 3 we see a reproduction of the suspension curves of a frog's heart after veratrin poisoning. (The heart was left in situ and the circulation of the blood was left intact; some drops of 1 per cent sol. acetas veratrini had been injected into the dorsal lymph sac about

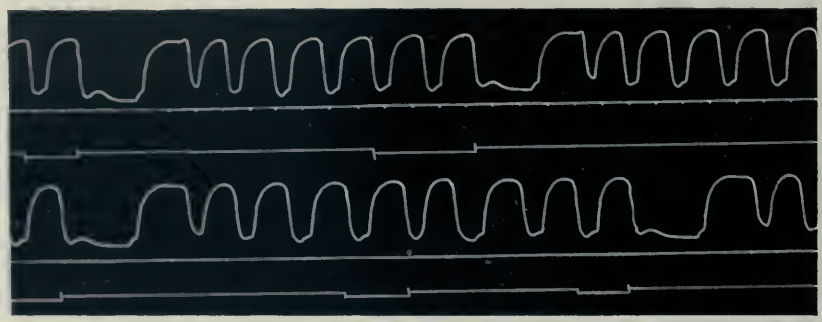

Fig. 3

10 minutes before.) At the first upward deflection of the signal an opening induction shock was given. After this we see an auricular systole represented in the suspension curve, which is not followed by a systole of the ventricle. Just as in the experiments described above, an extrapause of the ventricle follows after this auricular systole. At the next upward deflection the same experiment was repeated in the upper row of curves with the same result. Now when measuring the curve we find that the auricular systole, which appeared a short time after each of the two stimuli, follows after the commencement of the preceding auricular systole with an interval of a sinus period. We therefore applied the extra stimulus in the auriculo-ventricular groove a short time before the commencement of a normal periodic auricular systole. At that moment the ventricle was apparently still refractory, as there did not appear an extrasystole of the ventricle. The auricles,

however, respond to the stimulus. The excitation wave now traverses the auricles from the auriculo-ventricular boundary in the direction of the sinus venosus.

But simultaneously, the periodic sinus impulse traverses the auricles in an opposite direction. The two excitations meet and rebound. At that moment the auricular systole is accomplished under the influence of two excitation waves, passing through the auricles in opposite direction. The excitation waves clash against each other and are annihilated. Now we understand that the auricular systole succeeding the extra stimulus, originates partly under the influence of the periodic sinus impulse and partly from the extra stimulus.[4]

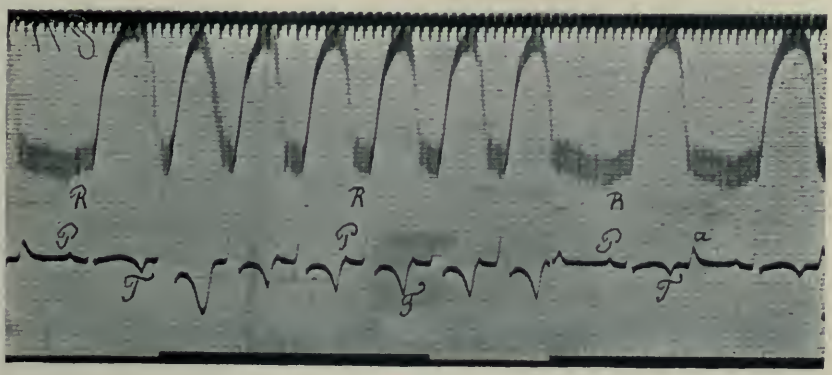

Fig. 4

It is also clear that this auricular systole cannot in this case be followed by a ventricular systole. In the lower curves, registered a little later, this experiment is repeated with the same result at the first and the third upward deflection of the signal. At the second upward deflection of the signal the stimulus is given a little later, so that then an extrasystole of the ventricle appears. In figure 4 are illustrated the suspension curves and the electrograms of a frog's heart after antiarin poisoning. Initially the ventricle pulsated in halved rhythm, which at the first upward deflection of the signal was changed into the normal rhythm of twice the velocity. At the second upward deflection of the signal another induction shock is administered in the auriculo-ventric-

[4] It goes without saying that it depends on the moment, at which the extra-stimulus is administered to which impulse the greater part of the auricular systole owes its origin. So, for instance, in figure 6 the two auricular systoles will arise for the greater part from the extra stimulus.

ular groove.[5] We see from the string curve that this stimulus is administered a short time after the P-deflection. At this moment the ventricle is apparently still refractory, so that an extrasystole of the ventricle is not evoked.[6] The auricles, however, do respond to the stimulus, so that these are now traversed at the same time by an excitation wave in a retrograde direction. This excitation wave, which traverses the auricles after the extra stimulus, encounters in the auricles the periodic sinus impulse, which was already on its way from the opposite side at the moment when the extra stimulus was given. Both excitation waves are then annihilated, so that no premature ventricular systole can follow and an extrapause of the ventricle manifests itself. Thereafter the normal ventricular rhythm is transposed into the halved rhythm.[7] It is beyond doubt that in this case the greater part of the auricular systole is owing to the periodic sinus impulse, because this impulse was already traversing the auricles at the moment when the extra stimulus was being administered. We have seen heretofore that at the moment when the extra stimulus in the auriculo-ventricular groove is administered, the ventricle must be refractory. To ensure the success of this experiment it will be well to lengthen the refractory stage of the ventricle.

In the two preceding experiments we have effected this lengthening by veratrin or by antiarin poisoning. We can now avail ourselves also of the fact that the refractory stage of the ventricle is lengthened by the postcompensatory systole. This is instanced in figure 5. It repre-

[5] The moment at which the extra stimulus is applied is marked by the signal and may also be seen from the string curve, which shows a small gap owing to a short swerving of the string.

[6] In the string curve we see directly after the stimulus, a small triangular deflection, which tells us that after all an extremely small part of the ventricle is contracted. We are safe to conclude that the sinus impulse can not rebound on this extremely small partial contraction, since, indeed, in the frog's heart the auricles are interconnected with the ventricle all along the auriculo-ventricular groove (auriculo-ventricular funnel). Similarly we see in figure 3 a slight difference in the magnitude of the deflections of the suspension curve, after the four stimuli which initiate the extra-pauses of the ventricle. Very likely also here an extremely small portion of the ventricle has been made to contract once or twice.

[7] I need not enlarge upon these transpositions of rhythm and the changes they involve for the ventricle electrograms. They were discussed by me in *Koninklyke Academie van Wetenschappen te Amsterdam Proceedings* XX, 696; (1917), 271 and 502. *Archives Néerl. de Physiologie*, iii (1918), 7 and 90. *Pflüger's Archiv*, Bd. 173, S. F. S. 1918.

sents the suspension curves of the auricles (lower curves) and of the ventricle (upper curves) of a frog's heart after bleeding. The stimulating electrode is applied in the auriculo-ventricular groove. At the downward deflection of the signal a closing shock is administered.[8] This gives rise to an extrasystole of the ventricle, which is followed by a compensatory pause. During the postcompensatory systole an opening shock is applied. Although this shock was administered at the commencement of an auricular systole just as the previous shock, the result is quite different. The refractory stage of the postcompensatory systole, namely, is lengthened, so that at the moment when the stimulus is applied the ventricle is still refractory and consequently presents no extrasystole. The auricles, however, do respond to the stimulus at the auriculo-ventricular boundary, so that consequently an excitation wave traverses the auricles in retrograde direction. This

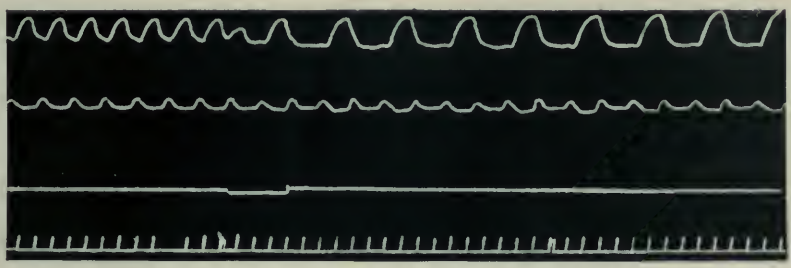

Fig. 5

excitation wave encounters in the auricles the periodic sinus impulse, so that both excitation waves are annihilated and no premature ventricular systole can follow. After the extrapause of the ventricle, thus originating, the following systole of the ventricle is extended and broadened. Now because this systole engenders a prolonged refractory stage of the ventricle, the ventricle is caught in the halved rhythm.[9] It is evident that the previously described experiments succeed only when the extra stimulus affects the auriculo-ventricular groove at a special moment.

If that moment coincides with the moment at which the periodic sinus impulse enters the auricles, the experiment will succeed. Success

[8] In this figure the closing induction shocks are not shut off and are announced by a downward deflection of the signal.

[9] These transpositions of rhythm in the bled frog's heart will be discussed in the following communication.

will even be achieved when the extra stimulus is applied somewhat later or earlier. In figure 4, e.g., at the second upward deflection of the signal, it was applied shortly after the P-deflection, therefore shortly after the periodic impulse had entered the auricles from the sinus venosus. In figure 6 the experiment succeeded twice through extra stimuli which were applied shortly before the P-deflection in the auriculo-ventricular groove. At the first upward deflection of the signal the extra stimulus was applied on the peak of the negative T-deflection, i.e., still before the P-deflection would be registered.[10] The excitation wave then traverses the auricles in a retrograde direction and encounters the periodic sinus impulse in the vicinity of the sinus venosus. The P-deflection, which otherwise would have revealed itself directly after the close of the T-deflection, does not appear now. The auricular systole is somewhat premature in this case and may

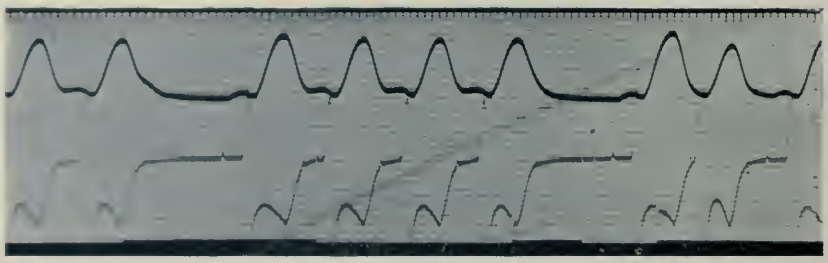

Fig. 6

still just be seen in the suspension curve in the last part of the ventricular diastole. It is obvious that this auricular systole is chiefly owing to the extra stimulus. At the second upward deflection of the signal the stimulus was applied a little before the peak of the T-deflection. The result is similar to that with the previous stimulus, viz., an extrapause of the ventricle.

If the extra stimulus is applied much later or earlier than the moment at which the sinus impulse enters the auricles, no extrapause of the ventricle will follow. If later the extra stimulus will affect the ventricle after the refractory stage and an extrasystole of the ventricle will ensue, followed by a compensatory pause. This is illustrated in figure 3, in the lower curves at the second upward deflection of the signal.

[10] In the electrogram we see the P-deflections appear directly after the close of the T-deflections.

Conversely, when the stimulus is given much earlier, an extrasystole of the auricles is originated, after which a systole of the ventricle follows at a prolonged a-v interval. An instance of this case is given in figure 6 at the third upward deflection of the signal.

At the first and the second upward deflection of the signal the extra stimulus was applied at the peak of the T-deflection or a short time before it, which resulted in an extrapause of the ventricle. At the third upward deflection of the signal, however, the extra stimulus was applied much earlier, viz., rather more than $\frac{1}{2}$ second before the peak of the T-deflection. It appears that the auricles respond already to the stimulus and present a complete extrasystole, but this retrograde excitation is not stayed in its course by the periodic sinus impulse in the auricles but probably in the sinus venosus. After this auricular extrasystole the excitation wave proceeds to the ventricle and induces it to contract.

Success of the latter experiment depends upon various conditions:

1. The extra stimulus is to affect the auricles after the refractory stage of these chambers.

2. After the artificial extrasystole the excitation wave is to reach the ventricle after its refractory stage.

Finally I wish to advert to the necessity of amplifying Engelmann's interpretation of the constant duration of the compensatory pause in connection with the present investigation. According to Engelmann the reason why, instead of the extrasystole a normal periodic ventricular systole has fallen out, is because the periodic sinus impulse reached the ventricle during the refractory stage of the extrasystole. The present research induces me to add that in some cases the periodic ventricular systole falls out because after the extra stimulus the excitation which proceeds also in retrograde direction, clashes upon the periodic sinus impulse, so that both excitations are annihilated.

When we thus amplify the interpretation of the duration of the compensatory pause, a fact becomes clear to me that had been known to me long since, namely that when an extra stimulus is given to the ventricle, we see in some of the experiments, during the extrasystole a P-deflection expressed in the electrograms, in others we do not. If the P-deflection is absent it is obvious that the periodic sinus impulse has not traversed the whole auricle, but has been stayed in its course by the excitation proceeding in retrograde direction, evoked by the extra stimulus.

SUMMARY

If we evoke an extrasystole of the auricles in the beginning of a ventricular systole, in some cases this extrasystole is not followed by a systole of the ventricle. This experiment succeeds only when, after the extrasystole of the auricles, the excitation wave reaches the ventricle before the close of the refractory stage. It was shown, that this experiment succeeds with more certainty if we lengthen the refractory phase by poisoning the frog's heart with veratrin, digitalis, antiarin or barium chloride. In the second place we know that the duration of a postcompensatory systole has increased and also the refractory phase of a postcompensatory systole has increased. Therefore does the experiment also succeed with more certainty if we evoke an extrasystole of the auricles in the beginning of a postcompensatory systole. In the third place we can evoke a prolonged pause of the ventricles in quite another way. We prolong the refractory phase of the ventricle and apply an induction shock in the auriculo-ventricular groove *toward the close of the diastole* and before the close of the refractory phase of the ventricle. Therefore an extrasystole of the ventricle does not appear but the auricles respond to the stimulus. The excitation wave traverses the auricles from the auriculo-ventricular groove in the direction of the sinus venosus. But simultaneously the periodic sinus impulse traverses the auricles in an opposite direction. The two excitation waves clash against each other and are annihilated. In this case the auricular systole is accomplished under the influence of two excitation waves passing through these two chambers in opposite directions. It is clear, that this auricular systole cannot be followed by a ventricular systole.

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RESEARCHES ON THE RHYTHM AND METABOLISM OF THE BLED FROG'S HEART

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Received for publication March 4, 1921

The following facts were among others stated by me in the pharmaco-physiological investigation I made on frogs' hearts, after I had poisoned them with veratrine, digitalis, antiarine or barium chloride (1).

1. The duration of the refractory stage of the ventricle muscle increases after the administration of each of these poisons, and so does likewise the a-v interval; at last the contractility of the ventricle muscle decreases.

2. As soon as the relative duration of the refractory stage

$$\left(\frac{\text{duration of the total refract. stage}}{\text{duration of a sinus period}}\right)$$

surpasses the value 1, suddenly or gradually the normal ventricle rhythm changes into the halved one.

a. The sudden halving of the ventricle rhythm comes about in the following manner: The duration of the refractory stage of the ventricle has increased during the normal rhythm of the ventricle for the reason that the ventricle muscle was not yet entirely restored at the beginning of every ventricular systole. What was still wanting to this restoration, was called by me the *residual refractory stage.*

The periodical refractory stage was added to it by every systole, so that the total refractory stage consists of two components. If now the relative duration of the refractory stage has become longer than 1, the next following ventricular systole falls away, and a protracted ventricular pause is the consequence. This protracted pause influences the two components in an opposite sense.

The ventricle muscle restores itself better, so that the residual refractory stage decreases. But after a protracted pause the next following systole of the ventricle is considerably enlarged, consequently the duration of the periodical refractory stage of the ventricle increases.

If now this increase of the duration of the periodical refractory stage surpasses the decrease of the residual refractory stage, then suddenly halving of the ventricle rhythm sets in.

b. The gradual transition to the halved ventricle rhythm however, takes place when the decrease of the residual refractory stage surpasses the increase of the periodical refractory stage. For, if this takes place, the normal ventricle rhythm continues after a protracted pause, till by accumulation the duration of the residual refractory stage causes again the falling away of a ventricular systole, and the normal ventricle rhythm is resumed again. So groups of ventricular systoles come into existence, which become gradually smaller and smaller, till in the end the halved ventricle rhythm is reached in this way.

3. Spontaneous alternations between the halved ventricle rhythm and the normal one occur frequently. The cause of these alternations lies in the fact that during the halved ventricle rhythm the katabolic index of the ventricle

$$\left(\frac{\text{duration of the total refract. stage of the ventricle}}{\text{duration of a ventricular period}}\right)$$

decreases again by restoration, till it has become less than one-half. Then the normal ventricle rhythm sets in again. In this twice as rapid ventricle rhythm[1] the katabolic index of the ventricle increases again under the influence of the small pauses of the ventricle and consequently the halved rhythm of the ventricle sets in again. So these alternations can repeat themselves several times.

4. By extra stimulation of the ventricle the halved ventricle rhythm can be artificially converted into the normal twice-as-rapid rhythm by the intercalation of one little ventricular systole. This proves that during the halved rhythm of the ventricle the sinus impulses that are not answered by the ventricle, did really reach this chamber of the heart, but rebounded on the yet refractory ventricle muscle.

The normal ventricle rhythm can likewise be converted into the halved one by extra stimulation. The enlarged postcompensatory systole fixed then the ventricle in the halved rhythm. I attributed these and many other results, not mentioned here, to the fact that an important factor of the action of the heart, viz., the refractory stage had been modified under the influence of the employed poisons. Its

[1] During the normal ventricle rhythm the katabolic index of the ventricle is equal to the relative duration of the refractory stage.

duration increased by veratrine, digitalis, antiarine and barium chloride. These poisons had no further possible mysterious actions for the results mentioned above.

SPONTANEOUS TRANSITION OF THE NORMAL INTO THE HALVED VENTRICLE RHYTHM OF THE NOT POISONED FROG'S HEART

The following observations made with regard to not poisoned frog's hearts afforded an unmistakable affirmation of the before-mentioned facts. *The before-mentioned sudden and gradual transition into the halved ventricle rhythm occurs likewise in the not poisoned frog's heart, the spontaneous alternations between the halved rhythm of the ventricle and the normal one can also be stated.* In figure 1[2] we give a reproduc-

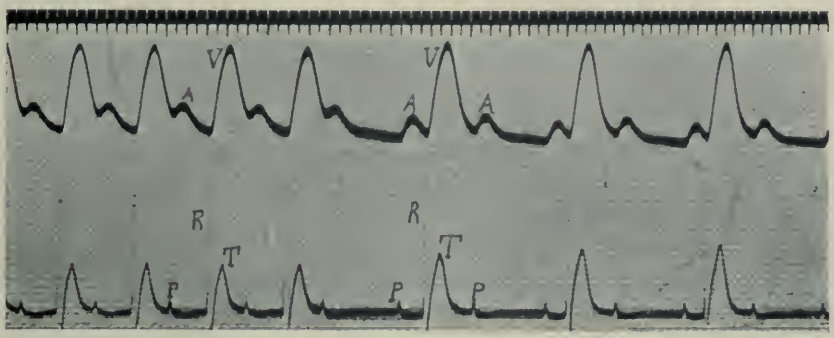

Fig. 1

tion of the suspension curves and the electrograms of a frog's heart (rana esculenta). More than an hour after the suspension this heart shows constantly repeated alternations between the normal ventricle rhythm and the halved one. I succeeded in photographing such a spontaneous alternation under simultaneous registration of the action currents.

This reproduction shows a great number of important details and affords a formal confirmation likewise for not-poisoned frogs' hearts, of the theoretical explanations communicated by me in former essays. In the figure we see suddenly appear the halved ventricle rhythm after four normal ventricular systoles. Three of these are still regis-

[2] Constantly one electrode was placed on the auricle and one on the ventricle in the following reproductions.

tered. I intend more explicitly to explain here the following details, which, in my opinion, are of interest for my subject.

1. As I indicated in my former investigations the a-v interval increases during the normal ventricle rhythm till the halving of the ventricle rhythm sets in. Afterwards the duration of the a-v interval decreases. The suspension curves of this figure show a much shorter a-v interval after the halving than before it. But the electrograms indicate these differences much sharper. The P-R interval increases still during the last four systoles. The first curve of the halved ventricle rhythm shows a much shorter P-R interval of the normal ventricle rhythm. The restoration of the ventricle muscle in the halved rhythm is even distinctly to be seen in these three first curves of the halved ventricle rhythm. The P-R interval of the second systole is shorter than that of the first, and that of the third still shorter than that of the second. We must attribute the shortening of the P-R interval after the halving to a shortening of the electric latent stage, as all sinus impulses reach the ventricle along the connecting systems and consequently the time of conducting along these has not in the least changed. It appears that this shortening still proceeds from the moment of the first ventricular systole of the halved rhythm.

2. The duration of the R-oscillation is, after the halving, shorter than before it; at the same time the height of the T-oscillation has increased. This duration of the R-oscillation is now, also, again shorter during the second systole than during the first, and at the third systole shorter than at the second. In concurrence with these facts the height of the T-oscillation increases from the first systole of the halved rhythm to the last one of the figure.

In the halved ventricle rhythm the conductivity through the ventricle is consequently better than in the normal twice-as-rapid rhythm of the ventricle. From the first systole of the halved ventricle rhythm, the conductivity still improves from systole to systole. The P-R interval and the duration of the R-oscillation consequently sustain alterations in exactly the same sense. We must attribute both these alterations to the changed metabolic condition of the ventricle muscle (katabolic index). This metabolic condition deteriorates in the normal ventricle rhythm. If now the rhythm of the ventricle suddenly halves the metabolic condition of the ventricle-muscles suddenly improves much, but also in the halved ventricle rhythm this improvement increases from systole to systole.

So this abrupt transition from the normal ventricular rhythm to the halved rhythm has originated in the following way. During the normal ventricular rhythm the duration of the residual refractory stage increases through accumulation from systole to systole. In the end the duration of the total refractory stage exceeds that of one sinus period. Then the next auricular systole is not followed by a ventricular systole and a prolonged ventricular pause arises. During this pause the duration of the residual refractory stage diminishes. This, of course, shortens the total refractory stage. But, at the same time, another influence is at work, which lengthens the duration of the total refractory stage. Its other component, viz., the periodic refractory stage increases in duration after the long ventricular pause. For after this pause the contractility of the ventricle augments. Now when, as in the present case, the increase of the duration of the periodic refractory stage exceeds the decrease of the duration of the residual refractory stage, the abrupt transition of the normal ventricular rhythm to the halved rhythm is brought about. This reproduction, which for the present moment will remain most likely exceptional among my material, afforded me an irrefutable confirmation of the theories I explained before. For the present I shall most likely be compelled in my further investigations to restrict myself to artificial transitions of poisoned frogs' hearts, and, when doing so, I shall, at the same time, register the action currents.

I am likewise in possession of beautiful examples of the slow transition to the halved rhythm of unpoisoned frogs' hearts. One example of these is reproduced in the figures 2, 3, 4 and 5.

The heart of a rana temporaria was suspended and soon showed group formation, because constantly one systole of the ventricle fell away. The groups grow gradually smaller, till groups of two and three systoles (figure 4) form the last transition to the halved ventricle rhythm (figure 5). We see during the groups the duration of the a-v interval increasing splendidly; again and again the ventricular systole sets in later in the auricular diastole, till one ventricular systole falls away. After this the interval is shortened again, to be protracted again in the same way during the following group. The ventricular systole of each first curve of the group commences in the figures 2, 3 and 4 close to the top of the auricular curve. The ventricular systole of each last curve begins at about the middle of the diastolic line of the auricular curves. This is the case with the large groups, but also with the little ones (bigeminus groups). Consequently in the beginning

more systoles of the ventricle are required than later to protract the a-v interval as much. The deterioration of the metabolic condition of the ventricle muscle is announced here by the formation of smaller groups. It is likewise clear that during the groups the metabolic condition of the ventricle muscle deteriorates, and improves again after

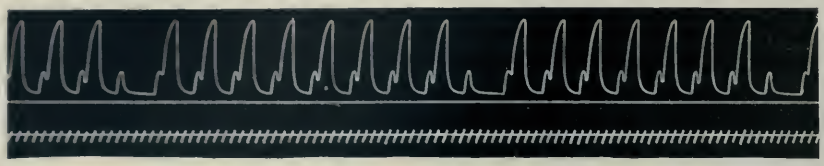

Fig. 2

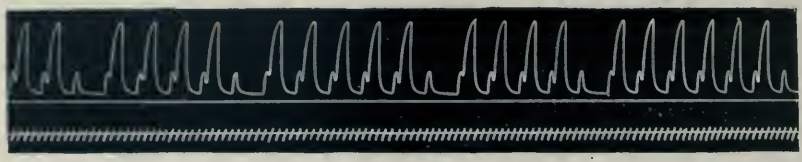

Fig. 3

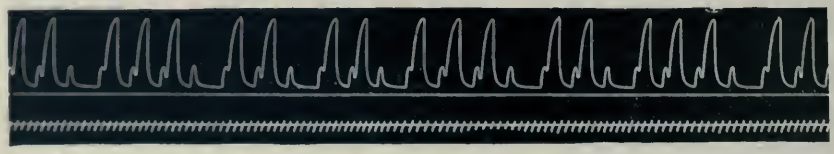

Fig. 4

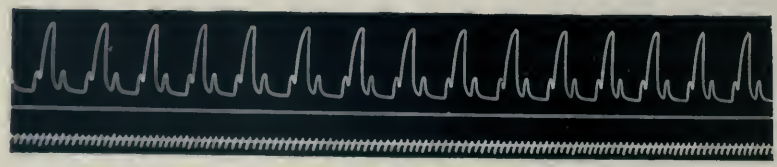

Fig. 5

a protracted pause. In my opinion we must here also attribute the protraction of the a-v interval again to a protraction of the latent stage of the ventricle muscle. It is the active contracting terminal organ, the ventricle muscle, the refractory stage of which increases during the groups and so does, at the same time, likewise the mechanical

latent stage. The increase of the refractory stage is here likewise caused by the increase of the duration of the residual refractory stage by accumulation. During the protracted pause after a group the decrease of the residual refractory stage surpasses the increase of the periodical refractory stage. In this way the constantly decreasing groups come into existence, which ends in the halved ventricle rhythm.

ARTIFICIAL CHANGES OF RHYTHM IN THE BLED FROG'S HEART

As has been shown in the preceding section, the halved ventricular rhythm may appear spontaneously in the bled frog's heart. This change may occur as soon as the duration of the total refractory stage of the ventricle exceeds the duration of one sinus period. Before the halved rhythm reveals itself spontaneously, we can halve the rhythm of the ventricle artificially, as appears from the following considerations. We call

$$\frac{\text{the duration of the total refractory stage}}{\text{the duration of a sinus period}}$$

the relative duration of the refractory stage.

When considering this fraction more carefully, we can say beforehand in what way the normal rhythm can be changed into a halved rhythm and the reverse, for if we take the relative duration of the refractory stage larger than one, the ventricle will pulsate in the halved rhythm. If, on the contrary, we take it smaller than one the ventricle will beat in the normal rhythm, in which every sinus impulse is followed by a systole of the ventricle. We can make the fraction greater than one by increasing the numerator or also by lessening the denominator. Now, in the case of a heart of which the total refractory stage is lengthened and which still beats in the normal rhythm, we can indeed prolong the total refractory stage so much as to make it outlast the sinus period. So we can make the fraction greater than one, as we have only to evoke an enlarged systole, whose refractory stage has been prolonged.

Now such an enlarged systole is the postcompensatory systole. When, therefore, we have lengthened the refractory stage of a ventricle (through poisoning or through bleeding) we evoke an extra-systole or extrapause of the ventricle. After the compensatory pause or extrapause the next ventricular systole is enlarged, while its refractory stage has been lengthened. Therefore, the subsequent sinus impulse

will be checked by this prolonged refractory stage; again, a prolonged pause ensues, and after this the next ventricular systole is again enlarged and has a prolonged refractory stage with all its consequences. Thus the ventricle is caught in the halved rhythm by the enlarged and broadened postcompensatory systole.[3] An increase of the duration of the refractory stage, i.e., an increase of the numerator of the above-mentioned fraction sufficed to bring about the ventricular halved rhythm. Another method producing the same result is heating the sinus venosus, which will increase the frequency of the sinus impulses and consequently decrease the duration of the sinus periods. The denominator of the fraction is diminished. When the ventricle pulsates in the halved rhythm, the relative duration of the refractory stage is greater than one. The fraction may then be made smaller by decreasing the numerator or by increasing the denominator.

The first may be effected by administering an extra stimulus to the ventricle during the diastole. Then an extra systole of the ventricle originates, which lasts much shorter than the ventricular systole from the ventricular halved rhythm. Therefore, the duration of its refractory stage is shortened and consequently the subsequent sinus impulse can elicit a ventricular systole. Owing to the short duration of the preceding ventricular pause, this systole will also be short, and accordingly will have only a short refractory stage. Therefore, here also the next sinus impulse is followed by a systole of the ventricle. Thus the ventricular halved rhythm is changed into the normal rhythm of double velocity. The extra stimulus during the halved rhythm may, however, be administered toward the end of the pause instead of during the diastole. Then the next sinus impulse reaches the ventricle during the diastole of the extrasystole and elicits a small ventricular systole. Whereas in the first case the normal ventricular rhythm was initiated by a small extrasystole, there now appears the normal rhythm under the influence of a sinus impulse, which reaches the ventricle in the diastole of an extrasystole and, therefore, yields a small systole. In both cases it was a *small ventricular systole with a short refractory stage*, that made the normal rhythm possible.

In the second place we can change the halved rhythm into the normal rhythm of twice its velocity by cooling the sinus venosus. Then the tempo of the sinus impulses is slackened by which the sinus periods are

[3] Not every postcompensatory systole is followed by a ventricular halved rhythm. This happens only when the refractory stage has been lengthened before by a disturbance of the metabolic equipoise.

lengthened. We will elucidate some of the above artificial changes of rhythm by some results obtained in experiments with the bled frog's heart.[4]

Let us first look at figure 5 of the previous publication.[5] The stimulating electrode is applied in the auriculo ventricular groove. At the downward deflection of the signal the ventricle receives a closing induction shock, which engenders an extra systole. At the end of the diastole of the postcompensatory systole, which has been enlarged, an opening induction shock is administered, which results in an extrapause of the ventricle.[6]

After the extrapause the first ventricular systole has increased still more in magnitude and in breadth, so that now the next sinus impulse rebounds on the refractory stage. The subsequent prolonged ventricular pause again causes an enlarged ventricular systole with a prolonged refractory stage.

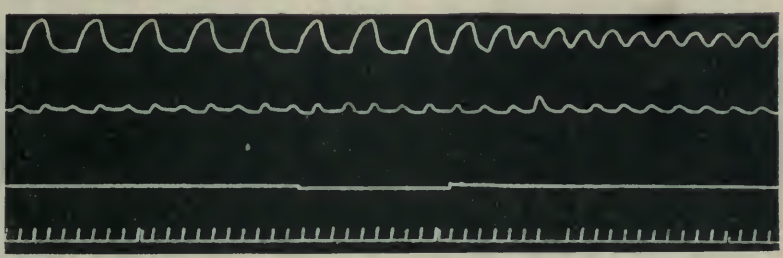

Fig. 6

Again the next sinus impulse does not result in a ventricular systole. Thus the ventricle, pulsating in the halved rhythm is, so to speak, caught in its own rhythm through the prolonged refractory stage. We can change this halved rhythm again into the normal rhythm of twice the rapidity, by eliciting a small ventricular systole. This happens in figure 6.[7] At the downward deflection of the signal an auricular extrasystole was evoked, after which the excitation reached the ventricle during the refractory stage. Consequently the rhythm of the ven-

[4] In all the figures of this publication the upper row represents the suspension curves of the ventricle, the lower row the suspension curves of the auricles.

[5] S. de Boer: On the artificial extra-pause of the ventricle of the frog's heart. This Journal.

[6] For the causes of this extra-pause I refer to the previous publication.

[7] Between figure 5 of the previous publication and figure 6 of this paper two ventricular systoles have not been reproduced.

tricle did not change here. However, at the upward deflection of the signal the stimulus was repeated toward the end of the pause. Now the auricles are refractory, but the ventricle responds to the stimulus with an extrasystole. After this the periodic sinus impulse reaches the ventricle at the end of the diastole so that a decreased systole of the ventricle ensues. This is accompanied by a short refractory stage so that also the subsequent sinus impulse again results in a ventricular systole. In this way every sinus impulse may be followed by a ventricular systole.

Figure 7 shows the suspension curves of a frog's heart, ten minutes after bleeding. The stimulating electrode is at the auricles. At the first downward deflection of the signal the auricles receive a closing shock which results in an extrasystole of the auricles followed by a compensatory pause.

It is evident that the ventricular rhythm is influenced only in this way that the next systole of the ventricle appears somewhat earlier.

Fig. 7

When, however, at the upward deflection of the signal the auricles receive the opening induction shock at an earlier moment of the auricular period, the result is quite different. After the thus excited extrasystole of the auricles, coinciding with the commencement of the ventricular systole, the excitation reaches the ventricle still in the latter's refractory stage.

After the compensatory pause of the auricles the next auricular systole is followed again by a ventricular systole. Thus arises an extrapause of the ventricle followed by an enlarged and broadened systole. Of this the refractory stage is prolonged, so that the next auricular systole cannot be followed by a ventricular systole. Again a prolonged ventricular pause arises, which is again followed by an enlarged systole of the ventricle. Thus the ventricle is caught in the halved rhythm by only one stimulus administered to the auricles. At the second downward deflection of the signal the auricles receive a closing shock toward the close of the pause, which evokes an extrasystole of these chambers. After this the next ventricular systole commences earlier.

The then following sinus impulse reaches the ventricle toward the close of the diastole and may,therefore, be followed by a small ventricular systole. This small ventricular systole now yields a short refractory stage. Therefore, the next auricular systole can be followed again by a ventricular systole, which, on account of the short duration of the preceding pause, is again small and short. For this reason the next auricular systole can again be followed by a ventricular systole. Thus by a single induction shock the halved rhythm of the ventricle is changed into the normal rhythm of twice the rapidity. Figures 6 and 7 show us that we are able to change the halved rhythm into the normal one by means of a single induction shock. Now the question arises why the ventricle does not take up the normal rhythm spontaneously. From the fact that the halved rhythm can be changed into the normal it indeed appears that the metabolic condition of the ventricular muscle enables the ventricle to beat with a double frequency. Still the ventricle persists in its halved rhythm unless we administer a stimulus at the right moment. The cause must be looked for in the magnitude and the long duration of the ventricular systoles of the halved rhythm. Every second sinus impulse rebounds on this prolonged refractory stage; the ventricle is caught in the halved rhythm and can escape from it only when, through an extra stimulus a small ventricular systole is evoked directly or indirectly.

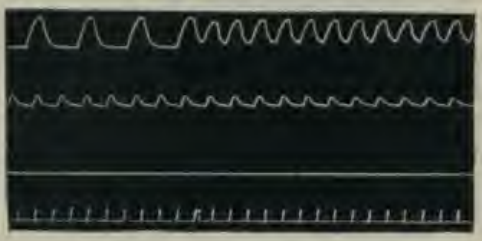

Fig. 8

When, however, the ventricle has been pulsating for some time in the halved rhythm, the ventricle gradually discards the residual refractory stage under the influence of the many prolonged ventricular pauses so that the total refractory stage is shortened after all. In this way the normal ventricular rhythm may yet return spontaneously. This is illustrated in figure 8.

The curves of this figure originate from the same frog's heart which produced the curves of figure 7.

When looking again at the ventricle curves of figure 5 of the previous publication and of figures 6, 7 and 8 of the present one we can state what follows:

As soon as the normal ventricular rhythm is changed into the halved rhythm the magnitude and the duration of the ventricular systole in-

creases. This increment then proceeds from systole to systole, so that the tenth systole of the halved rhythm is much greater than the fifth, which again in its turn is greater than the first. This increment of the magnitude of the ventricular systole is brought about by an increase of the maximum diastole and, at the same time, by an increase of the maximum systole. It will be seen, then, that the ventricular muscle recovers during the halved rhythm and that this recovery proceeds under the influence of an increase of long ventricular pauses. The reverse will be observed after the change of the halved rhythm into the normal. The ventricle is then in a good condition owing to the preceding halved rhythm. Directly after the change into the normal rhythm, the magnitude of the ventricular systoles has decreased. But under the influence of the frequent recurrence of short ventricular pauses the magnitude of the ventricular systoles lessens more and more. This lessening regards the maximum diastole as well as the maximum systole. An intermediate form between the normal ventricular rhythm and the halved rhythm is the ventricle alternation.

We can change the normal ventricular rhythm into the alternation and this again into the halved rhythm, as illustrated in the following figures, derived from the same frog's heart. The curves of figure 9 were taken five minutes after the bleeding. The ventricle was then pulsating in the normal rhythm; at the first deflection of the signal the auricles received an induction shock resulting in an extrasystole of these chambers which was followed by a small systole of the ventricle. At the second deflection of the signal again an auricular extrasystole was evoked in the beginning of the postcompensatory systole. Thereafter the excitation reaches the ventricle during the refractory stage so that an extrapause of the ventricle ensues. Then the first ventricular systole is very much enlarged. This enlarged ventricular systole introduces an alternation of the ventricle. (Similarly in our previous experiments the halved rhythm was brought about by an enlarged systole.) After some time this alternation changes spontaneously into the normal ventricular rhythm with systoles of the same magnitude. At the third deflection of the signal again an extrasystole of the auricles is evoked, followed by a small ventricular systole. After the enlarged postcompensatory systole again the ventricle alternation arises.

The curves of figure 10 were taken about one minute after those of figure 9, a short time before the alternation had been elicited experimentally. It still exists at the commencement of the figure. At the first deflection of the signal the auricles are excited to an extrasystole

by an induction shock in the beginning of a large ventricular systole. After this extrasystole the excitation reaches the ventricle during the refractory stage so that no ventricular systole follows; an extra pause of the ventricle does follow. After this extrapause the first ventricular systole is enlarged again so that the next sinus impulse reaches the ventricle during the refractory stage. Owing to this the next pause of the ventricle is again prolonged with the ordinary consequences. In this way the ventricular halved rhythm is brought about artificially.

At the second deflection of the signal again an extrasystole of the auricles is evoked in the beginning of a ventricular systole. Because

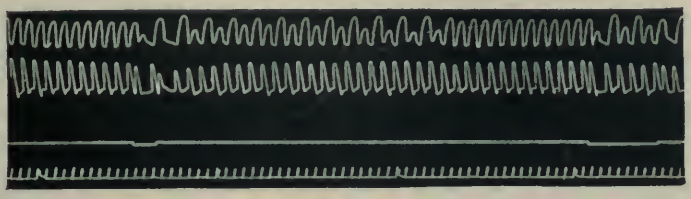

Fig. 9

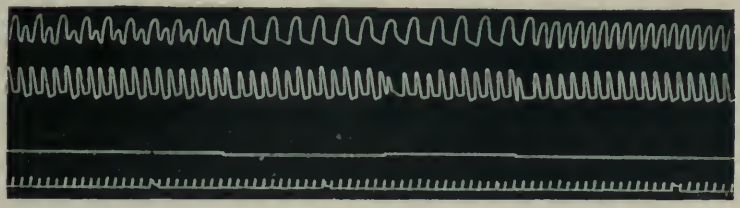

Fig. 10

hereafter the excitation reaches the ventricle during the refractory stage, the halved rhythm of course continues.

At the third deflection of the signal, however, an extrasystole of the auricles is evoked after the close of a ventricular systole. After this the excitation reaches the ventricle toward the end of the pause so that a premature ventricular systole follows. Now because this ventricular systole is premature the next sinus impulse reaches the ventricle after the close of the refractory stage, so that a small systole of the ventricle can follow. This systole is small on account of the short duration of the preceding pause and, therefore, causes a short refractory stage. For this reason also the following sinus impulse is

again followed by a ventricular systole, which also is small. In this way the normal rhythm of the ventricle is restored.

In the above we have given some instances of changes of rhythm in the bled frog's heart. We could enforce at will any given rhythm upon the ventricle by evoking *one* ventricular systole of a certain magnitude and duration.

ARTIFICIAL AND SPONTANEOUS CHANGES OF RHYTHM WITH SIMULTANEOUS REGISTRATION OF THE ELECTROGRAMS

It is exceedingly interesting to register cardiac electrograms during the artificial and spontaneous changes of rhythm. It affords us an opportunity of studying in the ventricular electrograms of one and the same frog's heart, the ventricular beats which occur after long and after short ventricular pauses. The influence of the duration of the pause immediately preceding on the form of the ventricular electrogram will appear from what follows:

A. Influence of the velocity of the conduction of excitation wave on the form of the ventricular electrogram. In a previous publication (2) I have set forth what was the influence of the velocity of the conduction of the excitation wave through the ventricle on the form of the ventricular electrogram. I could do this by slowing the conduction of the excitation wave in the same frog's heart. I thus obtained of one and the same frog's heart ventricular electrograms with rapid and with slow conduction of the excitation wave through the ventricle. Our procedure was threefold:

1. In the first series of experiments the electrograms of each frog's heart were registered while the circulation of the blood was left unimpaired. Subsequently a toxic dose of digitalis or of antiarin was given subcutaneously. With intervals of some minutes the electrograms were taken, until the ventricular rhythm began to be halved. Under the influence of the drug the velocity of the conduction of the excitation wave through the ventricle lessened. This led to the following alteration of the electrogram: α The R-deflection got broader; β the T-deflection was altered in a negative sense (a positive T-deflection became smaller, or changed into a negative one, a negative T-deflection became larger); γ the connecting line between the R-, and the T-deflection was lowered. These three changes made themselves more evident as the poisoning proceeded, i. e., as the velocity of the conduction of the excitation wave through the ventricle decreased more and

more. Then the rhythm of the ventricle was halved. During the halved ventricular rhythm the ventricular pauses have been enlarged. For this reason the excitation wave is sent through the ventricle at a quicker rate. This acceleration manifests itself directly in the form of the ventricular electrogram. The R-deflections narrow, the T-deflections are modified in a positive sense (the negative T-deflections become smaller or turn into positive T-deflections), the connecting lines between the R- and the T-deflections rise.

2. In the second series of experiments the normal ventricular rhythm was, after poisoning with digitalis or antiarin, transposed through one induction shock to the halved rhythm and this again to the normal one. In this way I procured records of the halved and the normal ventricular rhythm of the same heart. At the same time the electrograms were registered to the following effect:

When the normal ventricular rhythm was transposed to the halved rhythm, the R-deflections were narrowed, the T-deflections were modified in a positive sense and the connecting lines between the R- and the T-deflections rose. If, on the contrary, the halved rhythm was changed into the normal one the R-deflections broadened again, the T-deflections changed in a negative sense and the connecting lines between the R- and the T-deflections were lowered. These two series of experiments go to show distinctly that a slowing of conduction of the excitation wave through the ventricle causes the following regular and typical changes in the ventricular electrogram; the R-deflection broadens; the T-deflection changes in a negative sense; the connecting line between the R- and the T-deflections falls. A clearer view still was given of these typical changes in the third series of experiments.

3. In this series we experimented on non-poisoned frog's hearts. Ventricular extrasystoles were excited in a more or less premature stage of the ventricular period. The earlier a ventricular extrasystole was incited in the ventricular period, the slower was the conduction of excitation through the ventricle during the extrasystole. The electrograms of the frog's hearts were registered simultaneously. Now, when the extra shock was administered to the basis of the ventricle an electrogram resulted, of which the R-deflection was broadened, the connecting line between the R- and the T-deflections was lowered and the T-deflection had been changed in a negative sense. These modifications manifested themselves all the more distinctly according as the extra shock had been administered at an earlier stage of the ventricular period. It goes without saying that here we can only compare the

electrograms of the more or less premature extrasystoles among themselves, and not with those of the periodic ventricular systoles, in order to realize the influence of the velocity of the conduction of the excitation wave on the form of the ventricular electrogram. For with the former the induction shock affects the ventricle at a special point of the exterior muscular layer and with the latter the excitation wave proceeds along the atrio-ventricular connecting system. If, therefore, we wish to know the influence exercised by the velocity of the conduction on the form of the ventricular electrogram we can compare the electrograms of ventricular extrasystoles, which have been incited at an early stage of the ventricular period with those of the ventricular extrasystoles that have been incited later. The result was that with the former the R-deflection had been broadened more, the connecting line between the R- and the T-deflections had fallen lower, the T-deflection had undergone a greater change in a negative sense, than with the latter. These changes, therefore, will make themselves more evident according as the extrasystole was incited at an earlier moment of the ventricular period.

Now when we administer an induction shock at the apex, the excitation wave traverses the ventricle during the subsequent extrasystole from the apex to the base. This circumstance causes the R-deflection to revert, as already established by Samojloff (3). The R-deflection has also broadened and the more so according as the induction shock occurred earlier in the ventricular period, i.e., according as the excitation wave proceeded through the ventricle at a slower rate. The T-deflection has changed in a positive sense (a negative T decreased or turned into a positive one, a positive T increased) and that the more intensely, according as the induction shock was given at an earlier moment of the ventricular period. The connecting line between the R and the T has risen, and that all the more according as the induction shock had been administered at an earlier moment of the ventricular period.

In the foregoing we have compared the electrograms of the extrasystoles after base and apex-stimulation with the electrograms of the periodic ventricular systoles. However, in order to observe the influence of the velocity of conduction of the excitation wave on the form of the ventricular electrogram we compared, after base or apex-stimulation, the electrograms of the extrasystoles among themselves, which had been incited at an earlier or later moment of the ventricular period. In the experiments I undertook by means of stimulation of the auricles,

I also could compare the electrograms of the subsequent premature ventricular systoles with each other and also with those of the periodic ventricular systoles. For when an auricular extrasystole has been incited, the excitation wave will proceed along the atrio-ventricular connecting systems to the ventricle and will enter the ventricle at the same points as with the periodic ventricular systoles.

From these experiments it appeared that after extra-stimulation of the auricles the electrograms of the subsequent ventricular systoles exhibited the following alterations:

1. The R-deflections had been broadened.
2. The T-deflections were changed in a negative sense.
3. The connecting lines between the R- and the T- had been lowered.

These alterations became more distinct according as the ventricular systole appeared at an earlier moment of the ventricular period, i.e., according as the excitation wave was conducted through the ventricle at a slower rate.

The electrograms of the postcompensatory systoles appearing after base, apex or auricle stimulation, are also essential to our subject. After the lengthened compensatory pause the excitation is accelerated during the postcompensatory systole on traversing the ventricle. Owing to this the electrograms of the postcompensatory systoles undergo the following alterations:

1. The R-deflections have narrowed.
2. The T-deflections have changed in a positive sense.
3. The connecting lines between the R- and the T-deflections have risen.

These changes are revealed more distinctly according as the compensatory pause lasts longer, i.e., according as the preceding extrasystole of the ventricle is incited at an earlier moment of the ventricular period. The rate of conduction of excitation during the postcompensatory pause is the greater the sooner the preceding extrasystole of the ventricle has been incited in the ventricular period. It will be understood that the first two series of experiments (intoxication with antiarin or digitalis) were performed on frogs' hearts that were still perfused with blood. Their results are therefore not quite reliable, since the varying infusion of blood may affect the form of the ventricular electrogram.

In the third series of experiments we used bled frogs' hearts as well as those in which the blood-circulation was left unimpaired. The changes described above occurred in either case. This result also

lent support to the experiments of the first two series. The joint results of the three series of experiments may, therefore, be summarized as follows:

Slowing of the conduction of the excitation wave through the ventricle causes:

1. The R-deflection to be broadened.
2. The T-deflection to be changed in a negative sense.
3. The connecting line between R and T to be lowered. These changes will be greater according as the excitation wave is conducted through the ventricle at a slower rate. Acceleration of the conduction of the excitation wave through the ventricle causes.
1. The R-deflection to be narrowed.
2. The T-deflections to be changed in a positive sense.
3. The connecting line between R and T to rise. These changes will be greater according as the excitation wave is conducted through the ventricle at a quicker rate.

Why these changes influence the form of the ventricular electrogram when the conduction of the excitation wave is slowed or accelerated, we can elucidate best by some diagrams.

Figure 11 illustrates a ventricular electrogram with a positive T-deflection. This electrogram is the product of interference of the basal negativity *a*, *b*, *c*, with the apical *e*, *f*, *g*. The apical negativity commences at *e*, by which the initial basal deflection is compensated. Thus arises the R-deflection. After this the two negativities counterbalance each other, when finally the positive T-deflection appears because the basal negativity outlasts the apical.

Now when the velocity of the conduction of the excitation wave through the ventricle diminishes, the apical negativity commences later after the commencement of the basal. The point *e* gets farther removed from *a*. The entire apical negativity is thrust over and even past the basal, so that at the end the apical negativity is still persisting, when the basal negativity has already terminated. The T-deflection is consequently negative[8] (see fig. 12). Before the slowing (see fig. 11) point *n* of the basal negativity interfered with point *n'* of the apical. These two points being equidistant from the position of rest, their resultant coincides with the position of rest. But after the slowing *n* interferes with *m'* which is farther from the position of rest, so that the resultant is seen to lie below the position of rest. After the slowing

[8] It is obvious that the positive T. is diminished when the slowing of the conduction of excitation wave is less pronounced.

this holds good for all points of the basal negativity curves, which then interfere with points of the apical negativity curve, which are all farther away from the position of rest. In consequence of this the connecting line between R and T is lowered (fig. 12). When, on the contrary, the velocity of the conduction of the excitation wave increases, *e* gets nearer to *a*, and the curve of the apical negativity shifts in the opposite direction, so that after the conclusion of the apical negativity the basal negativity continues. This causes an enlargement of the positive T-deflection. After this acceleration every point of the basal negativity curve interferes with a point of the apical negativity curve, which lies nearer to the position of rest, so that the connecting line between R and T rises. (See fig. 13.)

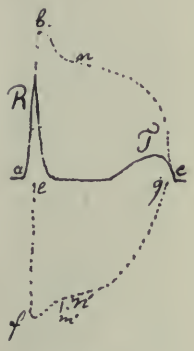

Fig. 11

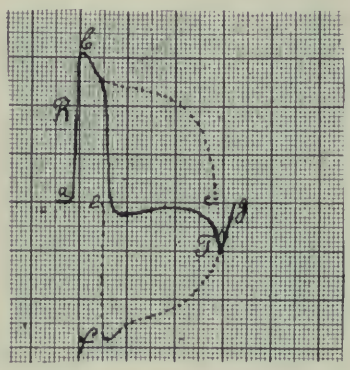

Fig. 12

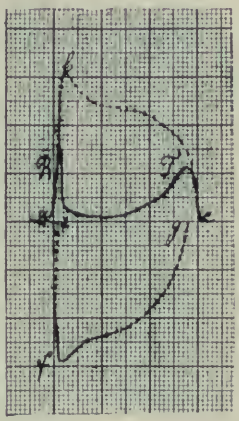

Fig. 13

After this investigation, which was in part carried out with poisoned frogs' hearts with unimpaired circulation some interest hinged about the study of the form of the electrogram of bled frogs' hearts, exhibiting analogous rhythmic disturbances. We will illustrate this by the following example:

Figure 14 shows the suspension curves (double suspension) and the electrograms of a bled frog's heart. The upper curves are derived from the suspended ventricle; then follow the auricle curves and under these the electrograms (derivation auricle-ventricular apex, tension of the string is such that 1 mv. yields a deflection of $1\frac{1}{2}$ mm.). In the upper illustration every third auricular systole is not followed by a systole of the ventricle, so that groups of two ventricular systoles are

engendered (at the end a group of three ventricular systoles can be seen). The first ventricular systole of every group appears after a long pause, the second after a short one, from which we see that during the first ventricular systole of every group the excitation wave traverses the ventricle at a quicker rate than during the second. This is brought out distinctly in the ventricular electrograms of the groups. The R-deflection of every second electrogram is much broader than that of every first, whereas the R-deflection of every second electrogram rises very slowly, that of every first electrogram rises abruptly.

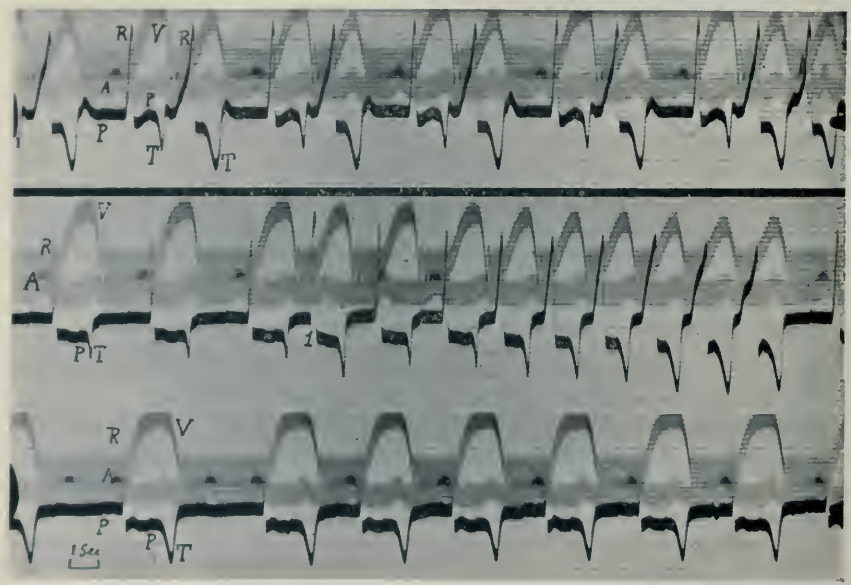

Fig. 14

The negative T-deflections of every second electrogram are much larger than of every first, and the connecting line between R and T is lower for every second electrogram than with every first.[9] Also the

[9] After every second ventricular electrogram occurs an approximately triangular upward deflection. Most likely this is the electric equivalent of an extremely small abortive third ventricular contraction. This view is favored by the following data: 1, these deflections commence after the conclusion of every third auricular systole; 2, now the deflections are more extensive, now again somewhat smaller, since now a larger, now again a smaller portion of the ventricular muscle is contracted; 3, in the trigeminus group at the end of the row the third R-deflection begins with such a triangular curve. The little positive peaks at the end of every first electrogram are of the same magnitude and occur no doubt with every first ventricular electrogram.

curves of the middle illustration are remarkable. Initially the ventricle pulsated in the halved rhythm so that with every two auricular systoles there is one ventricular systole. At *1* an induction shock was administered to the ventricle which generated an extrasystole, which is succeeded by a second. Now because the last mentioned extrasystole commences at an earlier moment of the ventricular period (directly after the P-deflection) it is only of short duration. The refractory stage of the extrasystole is also of shorter duration than that of any preceding ventricular systole of the halved rhythm. This is why the next auricular systole can be followed again by a systole of the ventricle, which in consequence of the short duration of the preceding ventricular pause is very brief again and is accordingly accompanied by a brief refractory stage. Therefore, also, now the next auricular systole can again be followed by a systole of the ventricle. Thus the halved rhythm is transposed by *one* induction shock to the normal rhythm of double velocity. This normal rhythm maintains itself during eight systoles and then again changes spontaneously into the halved rhythm of the ventricle. This transition takes place because during the normal ventricular rhythm the ventricle cannot restore itself sufficiently in the brief ventricular pauses. So a residual refractory stage remains after every ventricular systole, so that after eight ventricular systoles this residual refractory stage has been prolonged to such an extent that the duration of the total refractory stage exceeds the duration of a sinus period. In that case the next auricular systole is not followed by a systole of the ventricle, and a lengthened ventricular pause is brought about. After this pause the first ventricular systole is enlarged, it lasts longer and has a long refractory stage. The subsequent auricular systole can, therefore, not be succeeded by a systole of the ventricle and again a prolonged ventricular pause arises with all its consequences. Thus the halved rhythm reveals itself again spontaneously.[10]

A comparison of the ventricular electrograms of the two ventricular rhythms is now of interest for our subject. It is obvious that during every ventricular systole of the halved rhythm the excitation wave traverses the ventricle at a quick rate under the influence of the long ventricular pauses. During the normal ventricular rhythm the ventricular pauses are of short duration, so that the excitation wave traverses the ventricle slowly. The ventricular electrograms of the halved rhythm display narrow R-deflections, the T-deflections

[10] In the figure we see only the R-deflection of the first systole of the halved rhythm.

are negative, the connecting lines between the R and T are lying below the position of rest. The ventricular electrogram of the first systole of the normal rhythm displays a broadened R-deflection, the negative T-deflection is enlarged and the connecting line between the R- and the T- has been slightly lowered. After this the R-deflection broadens from systole to systole; the longer the normal rhythm of the ventricle maintains itself the slower is the rise of the R-deflections. In addition to this the negative T-deflections are getting larger and larger; the connecting lines are lowered more and more every time. So during the normal ventricular rhythm we see that the velocity of the conduction of the excitation wave decreases from the first to the eighth systole. The larger the number of preceding brief ventricular pauses, the slower is the rate at which the excitation wave traverses the ventricle.

During the halved ventricular rhythm the apical negativity surpassed already the basal negativity at the end of the electrogram. Therefore, already here, the T-deflection was negative. But if the velocity of the conduction of the excitation wave through the ventricle diminishes increasingly, the apical negativity continues longer and longer after the conclusion of the basal negativity. Thereby the ventricular electrogram is broadened more and more, and an increasingly greater part of the apical negativity still persists after the conclusion of the basal. This induces a longer duration of the ventricular electrograms. The last ventricular electrogram of the normal ventricular rhythm lasts much longer than the first. Since the frequency of the sinus impulses remains the same, the electric ventricular pause decreases progressively. It can be seen, therefore, that during the normal ventricular rhythm, the time during which the string remains in the position of rest between two electrograms is becoming shorter every time. Together with the progressive decrease of the velocity of the conduction of the excitation wave in the normal ventricular rhythm, this figure also shows a progressive decrease of the contractility of the ventricular muscle. Both the progressive decrease of the contractility and the progressive decrease of the rate of the conduction of the excitation wave are a direct consequence of the progressive deterioration of the metabolic condition of the ventricle. The greater the number of preceding brief ventricular pauses, the worse the metabolic condition becomes. We have also observed heretofore that after the transition from the normal ventricular rhythm to the halved rhythm, the contractility of the ventricle in this halved rhythm increases from systole

to systole. Therefore several long ventricular pauses are wanted to make the ventricle resume its contractility entirely. This also seems to be the case with regard to the velocity of the conduction of excitation, for if we consider the R-deflection of the first ventricular systole of the returned halved rhythm more narrowly, it is evident that this deflection has indeed become much narrower than that of the preceding normal ventricular rhythm. The immediate recovery of the rate of the conduction of the excitation wave through the ventricle after one long ventricular pause is, therefore, not questionable. Besides also the R-deflection rises quickly. Now in comparing the breadth of this R-deflection with that of the R-deflections of the halved ventricular rhythm with which this registration began, it appears that this R-deflection is no doubt about twice or three times as broad. It is evident, therefore, that after this single prolonged ventricular pause the velocity of the conduction of the excitation wave has by no means regained its optimum. It should seem, therefore, that for this as for the contractility several prolonged ventricular pauses are required. That after one prolonged ventricular pause the conduction of the excitation through the ventricle cannot recover its optimum, the upper illustration tends to show. After a prolonged ventricular pause the R-deflection of the first systole of every group has, indeed, become narrower, but is of about the same breadth as the last R-deflection of the second illustration. Here we fail to see after one prolonged pause a recovery of conductivity up to its optimum. From these two illustrations it is evident that in consequence of a slowing of the conduction of excitation through the ventricle:

1. The R-deflection broadens.
2. The T-deflection is modified in a negative sense.
3. The connecting line between the R and the T is lowered. These modifications will become more pronounced, according as the velocity of the conduction of excitation through the ventricle diminishes.

Hofmann (4) and Mines (5) believe that the duration of the ventricular electrograms affords an index of the contractility. The latter increases with a longer duration of the electrogam. Now, when measuring the curves of figure 14 we arrive at another conclusion. In the sets of two curves of the upper row the contraction of every first ventricular curve is the greatest, but the duration of the electrogram of every second curve far exceeds that of every first. A similar result is seen when measuring the second row of curves. In them the contractility decreases considerably during the normal ventricular

rhythm but the duration of the ventricular electrograms increases. Nay, the duration of the last ventricular electrogram even exceeds that of the ventricular electrograms of the halved ventricular rhythm with which this row commences. It follows that we cannot take the duration of the ventricular rhythms as an index of the contractility. The duration of the ventricular electrograms of every second curve of the groups of the upper row has increased so much because the velocity of the conduction of excitation through the ventricle has decreased considerably. Consequently the apical component of the ventricular electrogram overlaps the basal so that the electrogram is broadened. The same holds for the ventricular electrograms of the normal rhythm of the second row of curves. The longer the normal ventricular rhythm maintains itself the more the velocity of the conduction of excitation through the ventricle will decrease, and the more the ventricular electrograms will broaden. The tempo of the auricular beats being constant, we can read the increase of the duration of the ventricular electrograms in the normal ventricular rhythm directly from the decrease of the duration of the electric pauses.

B. The optimum of velocity of the conduction of the excitation wave through the ventricle. The lowermost illustration of figure 14 begins with the 3–1 rhythm: with every three auricular systoles there is one systole of the ventricle. After two ventricular systoles this 3–1 rhythm changes spontaneously into the halved rhythm of the ventricle. Now, when comparing the suspension curves of the ventricle in these two rhythms with each other we see that the contractility in these rhythms does differ. It is well known already that with a certain frequency, after artificial stimulation of heart-preparations that have been standing for some time, the cardiac muscle attains the optimum of contractility. (Hofmann (4) Mines (5).) A decreased and an increased frequency produce a decrease of contractility. From the lowermost row of curves it appears that, for this heart, the optimum of frequency of the ventricle is not yet reached with the halved and probably with the 3–1 rhythm. Acceleration of the pulsations decreases the contractility as will be seen from the middle illustration.

In this figure no slowing of the ventricular pulsations occurs that exceeds the 3–1 rhythm. So nothing can be said about it regarding this heart. True, the optimum of frequency has been reached probably during the 3–1 rhythm.[11]

[11] The height of contraction in either (2–1 and 3–1) rhythm is the same, but the duration of a ventricular systole of the 3–1 rhythm is longer than that of the halved rhythm.

Now the ventricular electrograms show that during the halved rhythm the velocity of the conduction of excitation through the ventricle has already reached its optimum. The longer ventricular pauses during the 3–1 rhythm do not increase this velocity any more, since the R-deflections in either rhythm are of the same breadth, the T-deflections of the same magnitude and the connecting lines between R and T are in both rhythms on the same level. We see then that the optimum of the contractility does not coincide wholly with the optimum of the velocity of the conduction of excitation wave through the ventricle[12]

C. On the relationship between the mechanic latent stage of the ventricle and the velocity of the conduction of the excitation wave through the ventricle. Gad's researches (6) have established that the mechanic latent stage of skeletal muscle is principally due to the mechanic relations of the registration. This has been demonstrated by Gad in the following way: He tied together two frog's muscles, the one above the other, and attached the terminal tendon of each muscle to a lever. Now the nether muscle was stimulated by an induction shock, so that it began to contract. The bottom lever now registered a shortening. The top lever however registered synchronously a lengthening. This was because the top muscle was extended through the contraction of the nether muscle. This finding Gad applied to one muscle, for an interpretation of the mechanic latent stage. For, when a muscle is affected by an induction shock at a definite point, the wave of contraction begins at that point. A short time after, a definite part of the muscle is contracted. This contracting portion now extends the flaccid portion of the muscle. As soon as the contraction-wave has proceeded so far that the contracting part exceeds the relaxing portion, the lever rises and the mechanical curve comes forward. It is evident, therefore, that the mechanic latent stage results from the mechanic relations of the registration. We may conclude from this also that the mechanic latent stage will last the longer, according as the excitation proceeds through the muscle at a slower rate. Now I found the same relations as those for skeletal muscle to exist also for the heart. Let us consider the two upper rows of curves of figure 14.

For index of the mechanic latent stage of the ventricular muscle we take the R-V-interval, the time elapsing from the commencement of

[12] In figure 14 the lower most row of curves was registered first, namely, some minutes after the bleeding. Then the middle one and finally the upper row, every time with an interval of some minutes. During the lowermost registration the string was slightly restless owing to a little disturbance in the compensation current.

the R-deflection to the commencement of the suspension curve, for when the R-deflection begins the excitation wave has no doubt already reached the ventricle. For index of the velocity of the excitation-wave through the ventricle we take the breadth of the R-deflection. We shall, therefore, compare the breadth of the R-deflections and the duration of the R-V-intervals during the sets of two systoles of the upper row and at the same time compare those of the halved rhythm and the normal rhythm of the second row.

Upper row of curves

Duration of the R-deflections of every first ventr. syst. $= \frac{1}{3}$ sec.
Duration of the R-deflections of every second ventr. syst. $= \frac{2}{3}$ sec.
Duration of the R-V-interval of every first ventr. syst. $= \frac{1}{4}$ sec.
Duration of the R-V-interval of every second ventr. syst. $= \frac{1}{2}$ sec.

From which we see that during every first systole succeeding a lengthened ventricular pause, the excitation wave is conducted through the ventricle twice as rapidly as during every second systole.

In accordance with this also the latent stage in every first ventricular systole has twice the length of that in every second ventricular systole.

Second row of curves

Halved ventricular rhythm.
Duration of the R-deflections $= \frac{1}{6}$ sec.
Duration of the R-V-interval $= \frac{1}{6}$ sec.

Normal ventricular rhythm

Duration of the R-deflections from the first to the last systole $\frac{6}{24}$ sec., $\frac{7}{24}$ sec., $\frac{8}{24}$ sec., $\frac{10}{24}$ sec., $\frac{12}{24}$ sec., $\frac{15}{24}$ sec., $\frac{16}{24}$ sec.

Duration of the R-V-intervals: $\frac{9}{24}$ sec., $\frac{8}{24}$ sec., $\frac{9}{24}$ sec., $\frac{10}{24}$ sec., $\frac{11}{24}$ sec., $\frac{11}{24}$ sec., $\frac{12}{24}$ sec. We see then that during the normal ventricular rhythm the excitation-wave proceeds through the ventricle at a slower rate than during the halved rhythm of the ventricle. The duration of the R-deflections increases from the first to the last systole of the normal ventricular rhythm, consequently the velocity with which the excitation wave traverses the ventricle diminishes. Accordingly the mechanic latent stage is of longer duration during the normal ventricular rhythm than during the halved ventricular rhythm. From the first ventricular systole of the normal ventricular rhythm down to the last there is an increase of the duration of the mechanic latent stage.

It follows, then, that *the duration of the mechanic latent stage of the ventricle increases with a decrease of the velocity of the conduction of excitation through the ventricle.*

I have expounded the relation between the velocity of the conduction of the excitation wave through the ventricle and the duration of the mechanic latent stage of the ventricle on the basis of the example illustrated in figure 14. I always found this relation in my previous researches, when ceteris paricus the metabolic condition of the ventricular muscle altered. For this I refer the reader to figures 4 to 18 (inclusive) of my communication in Pflüger's Archiv der Physiologie Bd. 173, Seite 78. "Ueber den Einflusz der Geschwindigkeit der Reizleitung auf die Form der Kammerelectrogramme."

D. On the electric latent stage of the ventricle. As observed before (page 190), from the fact of the transposition by means of one induction-shock from the halved rhythm to the normal we conclude that during the halved ventricular rhythm, also every sinus-impulse, not responded to by the ventricle, reaches the ventricle along the atrio-ventricular connecting systems. Because all sinus impulses reach the ventricle, a lengthening of the P-R interval during the normal ventricular rhythm can not be ascribed to a lengthening of the time of conduction through the auricles and the atrio-ventricular connecting systems, but to a prolongation of the electric, latent stage of the ventricle.

However, during the normal ventricular rhythm the P-deflections coincide with the ventricular electrograms and have become invisible. We, therefore, determine the A-R intervals (the time elapsing between the commencement of the auricular systoles and the beginning of the R-deflections). Duration of the A-R interval during the halved ventricular rhythm = $\frac{1}{2}$ second. Duration of the A-R intervals during the normal ventricular rhythm: of the first ventricular systole = $\frac{1}{2}$ second, of the last ventricular systole = $\frac{9}{12}$ second. It is evident, therefore, that the electric latent stage of the ventricle during the halved ventricular rhythm is as great as during the first systole of the normal ventricular rhythm. From the first systole of the normal ventricular rhythm the electric latent stage of the ventricle increases down to the last systole. We see, however, that this increase ($\frac{1}{4}$ second) is much smaller than the increase of the mechanic latent stage ($\frac{1}{2}\frac{3}{4}$ second).

SUMMARY

The results of our investigation are as follows:

1. After bleeding the frog's heart the halved ventricular rhythm may come forth spontaneously. This transition may occur abruptly or gradually through group formation. The causes of sudden and slow transition were ascertained.

2. A short time before the ventricular rhythm is halved, the metabolic condition of the ventricular muscle is deteriorated. This deterioration reveals itself *a*, in a lengthening of the refractory stage; *b*, in a slowing of the conduction of the excitation wave through the ventricle; *c*, in a lengthening of the a-v interval; *d*, in a decrease of the contractility.

3. After the ventricular rhythm has changed into the halved rhythm the metabolic condition of the ventricular muscle improves under the influence of the lengthened ventricular pauses. However, owing to the increase of the contractility and the longer duration of the ventricular systoles, the duration of the refractory stage does not decrease. The excitation is led through the ventricle at a quicker rate, the a-v interval is shortened.

4. The halved ventricular rhythm may be transposed by one induction shock to the normal, which is twice as rapid, when the shock is applied in the diastole. The normal rhythm returns again with the little extrasystole with its brief refractory stage. The normal ventricular rhythm can also return, when toward the end of the ventricular pause an extrasystole is called forth. Then the first sinus impulse incites in the diastole of this extrasystole a short ventricular systole with a brief refractory stage. This short ventricular systole can also induce the normal ventricular rhythm.

5. The normal ventricular rhythm may be transposed to the halved rhythm by calling forth an enlarged ventricular systole. This enlarged ventricular systole is obtained by inciting an extrasystole of the ventricle. Then the postcompensatory systole is broadened and has a lengthened refractory stage. Therefore it can induce the halved rhythm.

6. The ventricular alternation is an intermediate form between the normal and the halved rhythm. The normal ventricular rhythm may be transposed to a ventricular alternation by inciting an enlarged systole. This ventricular alternation may again be transposed to the halved rhythm by calling forth a ventricular systole, which is larger than the large systole of the alternation.

7. A ventricular systole of a definite magnitude can therefore induce a definite ventricular rhythm.

8. The influence of the rapidity of the conduction of the excitation wave on the form of the ventricular electrogram was observed.

9. After artificial transposition of the halved ventricular rhythm to the normal rhythm the contractility and the velocity of the conduction of the excitation wave gradually decrease from the first systole.

10. After transposition of the normal rhythm to the halved rhythm the contractility and the velocity of the conduction of the excitation wave increase from systole to systole.

11. With a definite slowing of the ventricular pulsations, the optimum of the contractility and of the conductivity was reached. These two optima did not coincide.

12. It was established that the mechanic latent stage of the ventricle is lengthened when the velocity of the conduction of the excitation wave through the ventricle diminishes. This happens in a greater measure according as the rate, at which the excitation wave is conducted through the ventricle, is slower.

13. The electric latent stage is also lengthened when the metabolic condition of the ventricle is deteriorated.

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STUDIES ON THE BRAIN STEM

V. Carbon Dioxide Excretion after Destruction of the Optic Thalamus and the Reflex Functions of the Thalamus in Body Temperature Regulation

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Received for publication June 4, 1921

In preceding reports (1) attention has been directed to the poikilothermous condition of birds that follows destruction of the optic thalamus. This is not due to temporary shock effects for it persists as long as the animals live. Such animals must be kept at an atmospheric temperature of about 30° C. to continue in good condition, but in this way we have kept them alive for 6 to 10 weeks after operation. The loss of ability to maintain a constant body temperature is not due primarily to a failure of the circulation for it has been found that if the animals are kept in a warm incubator the arterial pressure shows little variation from that of the homothermous decerebrate bird with thalamus intact. This suggested that possibly there might be some depression of the thermogenic mechanism and we have therefore carried out determinations of the carbon dioxide elimination in pigeons rendered poikilothermous by decerebration and cauterization of the optic thalamus. There is of course the uncertainty as to how strictly the carbon dioxide determination alone can be considered an index of heat production but it was assumed that with uniform conditions of diet or starvation it would serve as a indicator of any gross changes.

Methods. A respiratory mask was devised which fitted over the head of the pigeon and the expired air was drawn through sulphuric acid to remove water and the carbon dioxide was absorbed by solid moist lumps of sodium hydroxide. The inspired air was drawn over sodium hydroxide to render it free from carbon dioxide.

The mask was a thin rubber cylinder made from a condum one end of which was slipped over the head and tightly sealed to the neck by 1 per cent collodion, the other end was tied over a T-tube through one

end of which the inspired carbon dioxide free air was drawn and through the other end the expired air passed to the absorption tubes. The pigeon and the entire apparatus except the absorption tubes were set in a constant temperature incubator. Air was drawn through the apparatus by light suction.

Practice soon indicated certain precautions that must be observed with the apparatus.

1. Very thin collodion must be employed, otherwise as it dries it may form a hard constricting ring which may lead to asphyxia or even kill the animals. This is due to occlusion of the neck veins.

2. Light suction must be employed otherwise there may be a similar interference with the venous draining of the head leading to respiration difficulties. This of course would be particularly noticeable with animals in which the skull had been opened as in these decerebrate birds. This effect was avoided in two ways: first, the very thin rubber membrane of which the mask was made, in itself acted as a flutter valve; and second, minimum suction was employed. The only obstructions to the free flow of air through the system were two broad shallow layers of concentrated sulphuric acid through and over which the expired air was drawn. Care was taken that the tubes of sodium hydroxide should not become plugged by deposits of sodium carbonate. That the system was physiologically efficient was indicated by the absence of any respiratory difficulty while the determinations were being made and the fact that they were made repeatedly on birds in which the skull had been opened. Failure to observe any one of these precautions led to difficulties of breathing, as we soon learned.

The pigeon was wrapped in a towel to keep it quiet and the incubator door closed so that the animal was in semi-darkness. By using only adult pigeons, of the common street variety, it was assumed that the body surface area was very near the same and readings are therefore given in units of body weight rather than in terms of surface area.

Results. As in all determinations of normal metabolic rate, the carbon dioxide output of normal adult resting pigeons was found to vary widely with two primary factors: (1) External atmospheric temperature, and (2), state of the digestive activities. The wide variations according to external atmospheric temperature quickly indicated the necessity of keeping the animal at a constant temperature. All readings have therefore been made with the bird in an incubator set at a temperature of 28°–30°C.

Variations according to the state of digestive activity were checked by starvation for at least 3 days before making determinations of carbon dioxide. How important this factor is for birds may be seen by table 1. Thus the average of twenty readings on three normal pigeons on unrestricted mixed diet is 1.88 mgm. of carbon dioxide per hour per gram body weight, in comparison with 1.16 in the starving animals, an increase of 62 per cent. It seems probable that a very great part of this increase is to be attributed to the muscular action of the relatively large muscle stomach or gizzard and part of course may be due to the dynamic action of the absorbed substances. This factor must be taken into consideration since removal of the optic thalamus in the birds nearly always in our experience leads to an immediate disturbance in the digestive tract and hence the carbon dioxide readings on these birds seem abnormally low unless the control animals have been starved for a sufficient time to insure a minimum of activity on the part of the grinding stomach.

TABLE 1

Normal pigeons; birds on unrestricted diet; incubator temperature 21–30°C.

BODY WEIGHT	BODY TEMPERATURE	ROOM TEMPERATURE	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.	°C.		*mgm.*	*mgm.*
250	41	29	6	444	1.77
270	40–41	27–30	8	485	1.80
266	39–40	27–30	6	548	2.06

With the following precautions taken uniformly as a routine part of the procedure, 1st, as to the use of the respiraion mask; 2nd, preceding starvation for 3 or 4 days; 3rd, atmospheric temperature of 28°–30°C.; 4th, the bird exhibiting no struggles or respiratory difficulties; it was found that the carbon dioxide output of normal birds per gram body weight per hour varied between the extremes of 0.98 and 1.38 mgm. with an average in eleven birds of 1.16 mgm. (table 2).

These are much lower values than those cited by Corin and Van Beneden (2) due to the fact that the atmospheric temperature and feeding conditions were not kept constant by these workers.

In the homothermous pigeon, either the normal bird or the decerebrate bird with cerebral hemispheres removed but thalamus intact, the carbon dioxide elimination varies inversely with atmospheric temperatures below 30°C. (fig. 1). Above 36°C., the carbon dioxide increases as heat polypnea ensues.

The rôle of the feathers in the regulation of body temperature was tested as follows. In two pigeons the feathers were clipped off the entire body so as to leave the skin bare and they were then put in an ice box at 10°–14°C. These animals were left there for 24 hours with readings of body temperature at frequent intervals. The curve of diurnal variations differed only very slightly from that of the normal bird—thus in the normal bird the diurnal variations run on the average from 39° to 41°C. In these birds with feathers removed the curve ran from 38° to 41.5°C. This maintenance of body temperature to almost the normal level for hours when exposed to cold in the absence of feathers, together with the tremendous increase in carbon dioxide excretion in

TABLE 2

Normal pigeons; 3 to 5 days starvation; body temperature 39–40°C.; incubator temperature 30°C.

BODY WEIGHT	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.	*mgm.*	*mgm.*
258	2	236	0.91
260	2	360	1.38
245	2	246	1.00
267	2	298	1.12
223	2	288	1.29
266	2	364	1.36
258	2	322	1.24
259	4	307	1.18
265	2	344	1.29
256	2	252	0.98
241	7	298	1.23

the bird with feathers intact when exposed to cold, indicate that in birds the primary factor in maintenance of body temperature against cold lies in increased heat production and protection against heat loss is a secondary factor.

Removal of the cerebral hemispheres with the thalamus left intact does not reduce the carbon dioxide output. Thus in a pigeon under standard conditions of temperature and starvation, from which the hemispheres were removed and a functional thalamus was indicated by *a*, the fluffed position of the feathers; *b*, decerebrate restlessness and *c*, a normal body temperature, the average of three determinations was 1.10 mgm. of carbon dioxide per gram body weight per hour in comparison with the average of normal birds of 1.16 mgm. Further-

more such a decerebrate bird responds to exposure to a cold atmosphere by an increased carbon dioxide production and to heat (35°C.) by "panting" (fig. 1). This confirms the findings of Corin and Van Beneden (2) that removal of the cerebral hemispheres alone does not alter the thermogenic ability of the bird or its ability to regulate its body temperature through wide variations of the atmospheric temperature.

The carbon dioxide output of seven poikilothermous pigeons was determined with the following precautions:

1. To avoid possible depression of metabolism due to the cerebral traumatism or to shock, readings have been made at time intervals of 2 to 40 days after the operation.

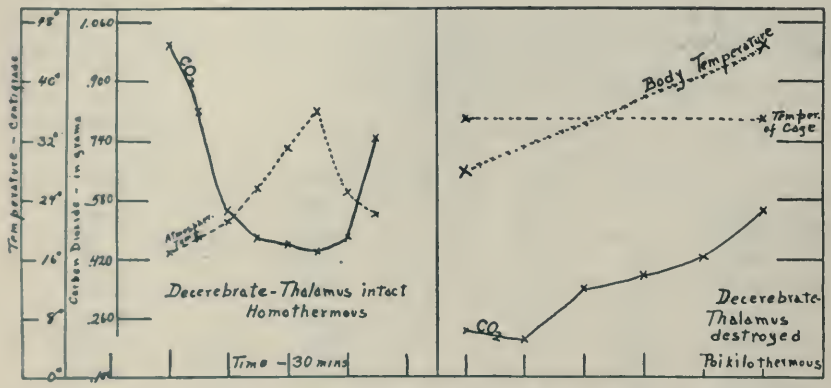

Fig. 1. Variations in carbon dioxide elimination in homothermous and poikilothermous decerebrate pigeons with variations of atmospheric temperature and body temperature. *a*) Curves to left, the homothermous pigeon. *b*) Curves to right, the poikilothermous pigeon.

2. The birds were starved from 2 to 4 days before the readings were made.

3. The attempt was made to keep the animal at an atmospheric temperature of 30°C., so as to keep the body temperature close to the normal value of 40°–41°C. It is not easy without constant slight changes of incubator temperature to keep the operated birds' temperatures within these limits.

4. The customary routine precautions in the use of the respiratory mask.

With these precautions the readings given in table 3 were obtained. It will be noted that in the pigeon in which the optic thalamus has been destroyed, if the body temperature is kept normal by being kept in a

warm atmosphere, the carbon dioxide elimination is very nearly the same as in the normal pigeon. As the body temperature is allowed to fall below 40°C., the carbon dioxide output also declines and varies directly with the body temperature between the wide limits of 27° to 44°C. (fig. 1). In this respect the results are analogous to the readings in cold-blooded animals (3). Freund and Grafe (4) find the same to be true of rabbits rendered poikilothermous by transection of the cervical cord. If the carbon dioxide output parallels the heat production of the body, these readings evidently indicate that normal heat production—thermogenesis—in the pigeon is not necessarily dependent on the

TABLE 3

Birds with optic thalamus destroyed; 2 to 4 days starvation; incubator temperature 27–30°C.

BODY WEIGHT	BODY TEMPERATURE	DAYS AFTER OPERATION	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.			mgm.	mgm.
220	38.0	4	2	228	1.03
235	39–40	4	4	296	1.25
211	39.0	2	2	342	1.62
215	40.0	5	2	242	1.12
284	38.0	4	3	244	0.85
226	41.0	2	2	219	0.96
232	38.0	3	2	203	0.87
250	37.5	48	2	249	1.00
251	27.0	5	2	219	0.87
220	36.0	4	2	196	0.89

temperature regulating centers of the thalamus but in this case is determined by the temperature of the tissues themselves, howsoever the tissue temperature be determined. At this point it should be emphasized that we are here dealing with lesions of the thalamus and not of the midbrain. Midbrain injuries in birds lead to violent muscular disturbances which have been rigidly excluded in this series. The absence of any appreciable midbrain injury is indicated by the complete absence of any visible disturbances of muscular coördination and the longer period of time that the bird with thalamic injury only may be kept living.

DISCUSSION

From the preceding we draw the conclusion that whatever may be the rôle of the thalamus in body temperature regulation, the quantity of heat production in the tissues is not of necessity dependent on this nerve center but is a function of the temperature of the tissues, howsoever that be fixed.

After removal of the optic thalamus in a pigeon, it differs from one with the thalamus intact in this respect: although the elimination of carbon dioxide is the same in both cases provided the body temperature in both is identical, the latter responds to atmospheric cold by an increase in carbon dioxide, the former by a decrease which varies directly with the extent of the atmospheric change.

Reviewing the preceding studies of this series we believe the following statement can be made as to the mode of action of the thalamus in regulating body temperature.

Thermogenesis is a function of the tissues not primarily dependent on this center but determined by conditions in the tissues themselves. Increased heat production when exposed to atmospheric cold is one of the primary essentials for the homothermous condition in the bird with protection against heat loss being a secondary factor. Although a minor factor, conservation of body heat against loss by radiation from the skin is undoubtedly effected to some extent by the feathers. The feathers are movable having a double set of muscles by which they may be either depressed or elevated. These muscles are innervated by sympathetic nerves (5). In the quiet normal bird exposed to cold the feathers are characteristically fluffed. Apparently this prevents or reduces contact of the cold with the skin, and reduces radiation from the skin although, so far as we know, no measurements on this point have been made. This elevation of the feathers to its full extent as seen in the sleeping bird or in the typical decerebrate bird is *dependent* on a functional thalamus. The change in position of the feathers after decerebration is an old observation noticed by earlier workers on the bird brain (Flourens, Schader, Munk). Becheterew (6) seems to have been the first to note specifically that the fluffed or depressed position of the feathers is determined by whether or not the thalamus is functional after decerebration. After destruction of the thalamus slight changes in feather erection occur dependent on body temperature variations (7) but never the complete elevation that is to be seen so characteristically either in the normal sleeping bird or in the bird with hemispheres only removed.

Heat dissipation in the homothermous pigeon is principally by evaporation or radiation from the lungs. When exposed to temperatures of increasing warmth there is a progressive reduction in heat production and at about 36°C., there is the onset of heat polypnea insuring a greater evaporation.

After destruction of the thalamus the following factors of regulation disappear:

1. Depression of the feathers against the body with resulting greater exposure of the skin to either cold or warmth.

2. Disappearance of polypnea when exposed to higher atmospheric temperatures and resulting hyperpyrexia of the animal.

3. Heat production in the tissues determined according to local tissue temperatures and not *inversely* by atmospheric temperatures.

4. Blood pressure varying directly with the body temperature and not constant independently of atmospheric temperature.

On the basis of these facts we advance the following view as a working hypothesis for further studies on the reflex functions of the thalamus.

Reflex augmentation of muscle tone or activity leading to increased heat production from stimulation of sensory nerve endings of cold is dependent on an intact thalamus. Heat polypnea or "panting" when exposed to excessive warmth is dependent not on the medullary centers alone but involves the thalamus. This may be either a reflex effect on the nerve endings or possibly one involving blood temperature. That the medullary centers alone are unable to carry out the muscular coördination required in "panting" is indicated by the work of various men on respiratory centers above the medulla oblongata as well as by the facts we report. Thus Nicolaides and Dontas (12) state that heat polypnea cannot be produced if the corpora striata be separated from the medulla. Such work will require a study of the neuro-muscular mechanism of "panting" in contradistinction to mere increase in rate or amplitude of breathing or hyperpnea. In birds reflex changes in the position of the feathers according to atmospheric temperature, namely, fluffing when exposed to cold and depression when exposed to heat, requires thalamic activity.

It is a well-known fact that painful stimuli (or noci-ceptive stimuli of Sherrington) induce reflex changes in striated muscle tone after separation of the spinal cord from the brain. Our results suggest that this is probably not true of milder stimulation of the cold and warmth nerve endings of the skin, but that sympathetic and muscle tone *reflexes*

from stimulation of the cutaneous temperature receptors, involve the thalamus as an essential part of the functional pathway. Or in the terms of Sherrington, reflex correlation between exteroceptive receptors of the skin (not including pain) and proprioceptive and interoceptive receptors, requires a higher complexity of integration than do the simple spinal reflexes of skeletal muscles induced by painful stimuli.

The only experimental work directly related to this view is that of Martin, Franklin and Hield and of Barbour. Barbour (8) finds that transection of the spinal cord in dogs abolishes the reaction of shivering when exposed to moderate depression of atmospheric temperatures. Martin and his co-workers (9) have studied vasomotor reflexes induced by warmth and cold applied to the skin of decerebrate rabbits. Unfortunately their work does not furnish crucial evidence either for or against the proposed view of thalamic functions since they did not differentiate between decerebrations with or without removal of the thalamus. But the view we propose may be the anatomical counterpart of their conclusion as to the vasomotor response being determined by the "quantity of nervous discharge" rather than to "stimulation of specific sense organs." The relatively large masses of grey matter constituting the thalamus, we suggest, are involved in the determinations of this "quantity of discharge" to which they refer. It is needless to add that this does not exclude a similar, possibly even a greater, action from the cerebrum itself.

We do not over-emphasize the importance of reflexes from temperature nerves in body temperature regulation for it seems to us that direct changes in the brain centers brought about by changes in blood temperature are probably more important as is emphasized in Barbour's work (10). Furthermore Ischensmid and Krehl (11) who, unknown to us until after this paper was written, have practically duplicated our work, using rabbits instead of pigeons, attempt to localize the centers of the thalamus necesary to body temperature regulation in the relatively small posterior, ventral and central grey regions.

SUMMARY

Removal of the cerebral hemispheres of pigeons, leaving optic thalamus intact, does not appreciably alter the output of expired carbon dioxide in resting starving birds, nor does it alter their ability to regulate body temperature against atmospheric cold by increased heat formation and against warmth, by polypnea.

In pigeons rendered poikilothermous by destruction of the optic thalamus, the carbon dioxide output varies directly with the body temperature variations. If the body temperature is set to a normal level by regulation of the atmospheric temperature, the output of carbon dioxide falls within the limits of variations of normal homothermous birds. After removal of the thalamus the pigeon does not respond to atmospheric cold by increased heat production nor to warmth (36°C.) by "panting" (polypnea).

It is suggested that reflex changes of skeletal muscle tone and of the sympathetic system induced by stimulation of the temperature nerves of the skin involve the thalamus as an esssential part of the functional pathway.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

IX. On the Relation of Stale Sperm to Fertility and Sex in Ring-Doves

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Received for publication June 6, 1921

The abnormal sex ratios observed in hybrid birds by Suchetet (1), Guyer (2), Whitman (3), Riddle (4), (5), (6), Smith and Haig-Thomas (7) and Phillips (8) justify any inquiry which may be made on either the spermatozoa or ova of these forms if the result assists in finding a factor, or in excluding what may by some investigators be considered a factor, in the causation of such abnormal sex ratios.

"Staleness" or "over-ripeness" of ova was one of the conditions associated with the very abnormal sex ratios which Hertwig (9) and Kuschakewitsch (10) obtained in experiments on sex determination in the frog. There is probably no good evidence that staleness of sperm is anywhere associated with abnormal sex ratios. In the extensive studies on sex in doves and pigeons being conducted by one of us it is desirable, however, to know whether stale germ cells are a complicating factor in the ratios obtained. The possibility of very stale ova of the bird being fertilized is excluded; but the possibility of the egg being fertilized by stale sperm certainly exists. It seems perhaps improbable that staleness of the sperm would influence sex in this material since in birds the differentiation of the germ cells with respect to sex almost certainly resides in the ova, not in the sperm; other and probably better reasons might also be stated. Nevertheless, the subject requires the test of experiment.

Moreover, the length of time during which spermatozoa may retain their fertilizing power in the oviduct of the female dove has apparently remained quite unknown. In breeding work it is sometimes necessary to know the duration of this period. The present work was so conducted that, whatever the result concerning the relation of stale sperm

to the sex ratio, we should definitely learn the age at which the stale spermatozoa of the dove lose their power to fertilize.

MATERIALS AND METHODS: The females used were nearly all hybrids of two closely related species of ring-doves —*Streptopelia risoria* and *St. alba*. Females 49 and B442 were hybrids involving the two last-named species and the Japanese ring-dove (*St. douraca*). At the beginning of these studies the thirteen females successfully studied were of ages varying from about 18 months to 5 years. Nine of the males were hybrids of the same kind as the females first mentioned above. Two (tables 1 and 3) were hybrids of the three above-named species. One pure-bred blond ring (*St. risoria*) was used (table 6). Normally, all of the above described pairs are fully fertile. In one case (table 11) a generic hybrid male was used which, so far as known, is not fully fertile in any cross.

Infertile eggs were occasionally produced by some of these birds before the experiment began. Most of the females used had been made to lay eggs with abnormal frequency. During the experiment a few eggs were obtained whose infertility is obviously due to the same factors which caused it prior to the beginning of the experiment.

Each pair was confined in a separate pen. The males were allowed their usual complete freedom of the pen with the female during (usually) 1, 2 or 3 days after the laying of a pair of eggs. In the latter part of the study this period was often shortened to 1 day or less. Thus in a few cases the separation was made after only 2 or 3 hours; for the shortest of such periods the birds were watched until one or more copulations had occurred. The male was then confined in a smaller cage placed within the pen belonging to the pair.[1] The date and hour of confinement of the male was noted. The time of "separation of the parents," as this is indicated in tables 1 to 11, has been reckoned from the hour of separation to the hour of the laying of the next following egg. It is practically certain that fertilization occurs, if at all, very soon after the egg leaves the ovary. Since in these doves ovulation occurs 40 to 44 hours[2] before the egg is laid it is clear that 1 day or slightly more should be deducted from the figures for staleness of

[1] The fact that female ring-doves require stimulation of a sexual nature for the production of mature eggs makes it advisable or necessary to leave the male in plain sight and as nearly in contact with the female as is possible and still prevent copulation. The period during which the birds were left together after the laying of a pair of eggs was intended to insure the beginning of growth in the next pair of ovarian eggs.

[2] Riddle, unpublished data.

sperm as given in the tables. On the other hand, in most instances it is not certain that one of the last cells liberated by the male is the one that fertilizes the egg. On the basis of chance it probably is usually a somewhat older sperm that accomplishes the fertilization, except in those cases in which all such older sperms have completely lost their power to fertilize or for which sperms from only a single copulation were available.

It sometimes happened that a female failed to produce eggs after the removal of the male; and a few females failed to lay eggs under these conditions in all of the trials made with them. These latter complete failures have not been tabulated nor otherwise considered. In other occasional failures—when eggs were not produced after a reasonable period of separation—the pair was reunited until eggs were again laid and the results of such fertilizations with normal sperm are given in parentheses in columns 3 and 6 of tables 1 to 11, and are summarized in a section of tables 12 and 13.

All of the eggs studied were incubated by pairs of birds kept for such a purpose. Fertility was doubtless somewhat reduced in four pairs of birds by the tuberculosis which developed in one or both parents during the course of the year utilized for this investigation.

Presentation of Data: *Stale sperm and fertility.* The records of each of the several pairs may first be examined with a view to learning the length of the period which sperm cells may remain in the female genital tract and retain their fertilizing power. The data indicate that this period was nearly the same for several of the pairs.

In table 1 it will be observed that all except 7 of the 63 eggs tested were fertile, and that for 6 of the 7 exceptions only very stale sperm was available for fertilization—6.2, 6.7, 7.7 (two cases), 7.9 and 8.7 days. In one case an egg was infertile although normal sperm was presumably available. Nine eggs with 7.7-day periods were fully fertile.

The pair whose record is given in table 2 was somewhat less fertile than the preceding pair. This female often refused to produce eggs when separated from the male. For this reason 16 eggs shown in the table were fertilized by normal sperm. Two of this group of eggs showed only slight development. Of the 46 eggs fertilized by stale sperm 16 were wholly infertile (3 others produced 1-day embryos or less). Nine of the 16 infertile eggs were associated with sperm of 7 to 13.7 days of staleness. One egg was fertilized 8 days after separation of the male. Three eggs produced at 9.7 to 18.7 days after separation of the male were wholly infertile. The fertilizing power of the stale

TABLES 1 AND 2

Effects of fertilization with stale sperm in females E361 (left) and 49 (right)

TABLE 1

Date of eggs	Days of separation of parents	Fertility and sex; remarks	Date of eggs	Days of separation of parents	Fertility and sex; remarks
11/25	(0)	(♀)	4/19	7.0	♀
11/27	(0)	(♂)	4/21	8.7	0*
12/4	5.0	♀	4/27	6.0	♂
12/6	6.7	♂	4/29	7.7	♂
12/13	5.0	♀	5/5	6.0	2-day
12/15	6.7	♀	5/7	7.7	0
12/22	3.3	?♀	5/13	6.0	♂
12/24	5.0	♂	5/15	7.7	♀
12/31	5.0	♂	5/21	6 0	♀
1/2	6.7	♀	5/23	7.7	♀
1/8	4.0	♀	5/29	6.0	♀
1/10	5.7	♂	5/31	7.7	♀
1/17	5.0	♀	6/6	6.0	6-day[3]
1/19	6.7	♂	6/8	7.7	3-day[3]
1/26	5.0	♂	6/14	6.2	0
1/28	6.7	♂	6/16	7.9	0
2/3	4.0	♂	6/21	4.3	♂
2/5	5.7	♀	6/29	6.0	♀
2/11	4.3	♂	7/1	7.7	♂
2/13	6.0	♂	7/6	4.3	4-day
2/19	4.0	♂	7/8	6.0	4-day
2/21	5.7	♂	7/14	6.0	♂
2/28	6.0	♀	7/16	7.7	♀
3/2	7.7	♂	7/22	6.0	♀
3/9	6.0	♂	7/24	7.7	Sl.[2]
3/11	7.7	0*	8/14	(0)	(♂)
3/17	5.0	♀	8/16	(0)	(♂)
3/19	6.7	♂	8/22	6.0	♂
3/25	5.0	♂	8/24	7.7	♀
3/27	6.7	0	9/14	(0)	(0)
4/2	5.0	♀	9/16	(0)	(♂)
4/4	6.7	♂			
4/10	5.0	♀			
4/12	6.7	♂			

TABLE 2

Date of eggs	Days of separation of parents	Fertility and sex; remarks	Date of eggs	Days of separation of parents	Fertility and sex; remarks
8/21	3.0	0*	4/9	6.0	♀
8/23	4.7	0	4/11	7.7	0
8/29	4.0	♂	4/20	8.0	Fert.[1]
8/31	5.7	1-day	4/22	9.7	0
9/6	4.0	Br.[1]	4/29	6.0	0
9/8	5.7	♀	5/1	7.7	0
9/15	5.0	♀	5/8	6.0	Sl[2]
9/17	6.7	♀	5/10	7.7	0
9/23	4.0	♀	5/19	8.0	0
9/25	5.7	♂	5/21	9.7	Br.[1]
10/1	4.0	♂	5/28	5.3	♀
10/3	5.7	♀	5/30	7.0	0
10/10	5.0	♀	6/5	4.0	♀
10/12	6.7	Br.[1]	6/7	5.7	♀
11/2	(0)	(Hat.)[4]	6/13	5.2	0
11/4	(0)	(Sl.)[2]	6/15	6.9	0
11/23	(0)	(Sl.)[2]	6/22	5.0	♂
11/25	(0)	(♀)	6/24	6.7	0
12/18	(0)	(♂)	7/2	7.0	♀
12/20	(0)	(♂)	7/4	8.7	Dis.[5]
1/8	(0)	(♀)	7/10	4.0	♀
1/10	(0)	(?♀)	7/12	5.7	♀
1/18	6.0	11-day	7/20	6.0	♀
1/20	7.7	0	7/22	7.7	Sl.[2]
2/8	(0)	(♀)	7/30	6.0	0
2/10	(0)	(♀)	8/1	7.7	Dis.[5]
3/1	(0)	(♂)	8/9	6.0	♀
3/3	(0)	(♀)	8/24	2.2	♀
3/10	5.0	♂	8/26	3.9	♀
3/12	6.7	4-day	9/7	12.0	0
3/19	5.0	?♀	9/9	13.7	0
3/21	6.7	13-day	9/16	(0)	(?♂)
3/31	(0)	(♀)	9/18	(0)	(♀)
4/2	(0)	(♀)			

*Signifies "not fertile."

[1] Egg was broken.

[2] Egg showed slight development

[3] Embryo killed by accident.

[4] Bird hatched; died, decayed before examination.

[5] Egg disappeared (almost certainly broken in nest).

TABLES 3 AND 4

Effects of fertilization with stale sperm in females B442 (top) and A684

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
1/27	(0)	(♂)	5/27	4.3	♀
1/29	(0)	(♂)	5/29	6.0	♀
2/4	4.0	♀	6/4	5.0	Some development
2/6	5.6	♀	6/6	6.7	♀
2/12	4.0	♂	6/13	6.0	♀
2/14	5.7	♂	6/15	7.7	2-day embr.; killed
2/20	4.0	♀	6/23	6.3	♂
2/22	5.7	♂	6/25	8.0	0*
2/28	4.0	♂	7/1	4.4	♀
3/2	5.7	♂	7/3	6.1	♀
3/8	4.0	♂	7/9	4.0	♀
3/10	5.7	♂	7/11	5.7	Egg broken
3/16	4.0	♀; 13-day embryo	7/17	4.0	?♂; 10-day embryo
3/18	5.7	Egg disappeared	7/19	5.7	Fertile; broken
3/24	4.0	♀	7/25	5.0	♂
3/26	5.7	♂	7/27	6.7	♀
4/1	5.0	♀	8/2	4.0	11-day embryo
4/3	6.7	♂	8/4	5.7	7-day embryo
4/9	5.0	♂; 13-day embryo	8/10	4.0	5-day embryo
4/11	6.7	♂; 13-day embryo	8/12	5.7	♂
4/17	5.0	♂	8/18	4.0	5-day embryo
4/19	6.7	♀	8/20	5.7	5-day embryo
4/25	5.0	♂; 12-day embryo	8/26	6.2	4-day embryo
4/27	6.7	5-day embryo	8/28	7.9	3-day embryo
5/3	4.3	♀	9/4	7.1	8-day embryo
5/5	6.0	6-day embryo	9/6	8.8	0
5/11	5.0	♂	9/13	(0)	4-day embryo
5/13	6.7	Egg disappeared	9/15	(0)	3-day embryo
5/19	5.0	Deformed (decayed)	9/22	(0)	(♂)
5/21	6.7	♀	9/24	(0)	(8-day embryo)[1]
3/15	8.0	0*	6/8	6.0	♀
3/17	9.7	0	6/10	7.7	0
4/3	5.2	♀	6/16	5.0	♀
4/5	6.9	♂	6/18	6.7	♂
4/11	4.0	♂	6/25	6.0	♂
4/13	5.7	♀	6/27	7.7	♀
4/19	4.0	♀	7/4	6.0	♀
4/21	5.7	♂	7/23	(16 or 0)[2]	(♂)
4/27	4.0	♀	7/25	(0)	(♂)
4/29	5.7	♀	8/1	5.3	♀

TABLES 3 AND 4—*Concluded*

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
5/6	6.0	♀	8/3	7.0	♂
5/8	7.7	♀	8/9	4.0	♀
5/21	12.0	0	8/11	5.7	♀
5/23	13.7	0	9/13	(0)	(♂)
5/30	5.0	♀	9/15	(0)	(♀)
6/1	6.7	♂; 12-day embryo			

* Signifies "not fertile."

[1] Probably most of the eggs laid at this period had shells that were too thin. The early death of these embryos was probably associated with these defective shells.

[2] The male was separated from the female 16 days before this egg was laid. He was again admitted 31 to 32 hours before the egg was laid. Normally the egg requires more than this amount of time to deseend the oviduct; it is also normally fertilized soon after entering the oviduct. It seems extremely probable, however, that this egg was fertilized by a fresh, not by a stale, sperm.

sperm cells of this pair was retained probably between 8 and 9 days. In only one other instance (table 8) in this study was an egg fertilized when the period of separation from the male was as much as 8 days.

Table 3 shows the result of testing 51 eggs with fertilization by stale sperm. The degree of staleness varied between 4 and 8.8 days. All eggs were fertile except the two which were laid after longest separation of the male, 8 and 8.8 days. One egg with a 7.9-day period was fertile. Many of the embryos of this record died early. This will also be noted in the records of three additional females which were used in the present study. These several records may properly raise the question whether fertilization by stale sperm is connected with the short life-term of the embryos. Data obtained from the other birds used in this study indicates, however, that no such connection exists; and a later special examination of the eggs produced by these birds has shown that not stale spermatozoa but inadequate egg-shells and some further deficiency or weakness of the ovum itself are associated with the early death of these embryos (16). No evidence was obtained in any part of this work which would indicate weakness or modified viability due to stale sperm parentage; the hatched young were, however, killed at an early age.

Table 4 gives the results of 27 tests with stale sperm. All eggs were fertile except 4 in which the degree of staleness exceeded 8 days (8 to

TABLES 5 TO 7

Effects of fertilization with stale sperm in females 79 (top), 744 (middle), and 192

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
3/17	(0 or 14)	(4-day embryo)	6/11	5.0	♂
3/19	(0)	(♀; 12-day embr.; killed)	6/13	6.7	♀
3/26	5.0	♂	6/19	5.0	Some (abnor.) devel.
3/28	6.7	♂	6/21	6.7	Egg broken
4/3	4.0	♀	6/27	5.0	♂
4/5	5.7	♀	6/29	6.7	0*
4/11	4.0	♀	7/6	6.0	♂
4/13	5.7	♀	7/8	7.7	0
4/20	5.0	♂	7/14	5.0	Abnor. vasc. area
4/22	6.7	♀	7/16	6.7	♀
4/29	5.0	4-day embryo	7/23	6.0	♂
5/8	6.0	Egg broken	7/25	7.7	0
5/10	7.7	4-day embryo	8/1	5.3	0
5/16	5.0	♀	8/3	7.0	0
5/18	6.7	*No gonads*; 14-day embr.	8/9	4.0	♂
5/24	5.0	Egg disappeared	8/11	5.7	♀
5/26	6.7	♀	9/13	(0)	(?♂)
6/2	6.0	♀	9/15	(0)	(♂)
6/4	7.7	♀			
7/4	4.0	Fertile; egg broken	8/13	4.0	♂
7/6	5.7	♂	8/15	5.7	♂
7/12	4.0	Slight (abnor.) devel.	8/21	5.0	Good vasc. area
7/14	5.7	♂	8/23	6.7	♂
7/20	4.0	Egg broken	8/29	6.2	Egg broken
7/22	5.7	♀; 11-day embryo	8/31	7.9	9-day embr.; killed
7/28	4.0	Some development	9/6	6.2	5-day embryo
7/30	5.7	♂	9/8	7.9	3-day embryo.
8/5	4.0	Slight (abnor.) devel.	9/14	(0)	2-day embryo
8/7	5.7	2-day embryo; abnor.(?)	9/16	(0)	(♂)
8/15	5.0	0*	2/10	4.0	♀
8/24	5.0	0	2/12	5.7	0
9/1	4.0	♀	2/18	4.0	♂
9/11	6.0	0	3/26	(0)	(2-day embryo)
9/19	4.0	0	3/28	(0)	(3-day embryo)
9/21	5.7	?♀; decayed	4/3	4.0	5-day embryo
9/27	4.0	Slight development	4/5	5.7	♀
9/29	5.7	♀	4/10	4.0	♀
10/5	4.0	♀	4/12	5.7	♀
10/30	(0)	(Slight development)	4/18	5.0	♀

TABLES 5 TO 7—*Concluded*

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
11/1	(0)	(♂)	4/20	6.7	9-day embryo
11/8	5.0	4 day embryo	4/26	5.0	♀
11/17	5.0	♀	4/28	6.7	Egg broken
11/26	5.0	♂	5/4	5.0	0
12/19	(0)	(0)	5/6	6.7	0
12/21	(0)	(♀)	5/12	5.0	♂
12/28	5.0	♀	5/14	6.7	♂
1/6	5.0	0	5/20	5.0	♀
1/8	6.7	0	5/22	6.7	♀
1/15	5.0	♀	5/29	6.0	♂
1/24	5.0	0	5/31	7.7	♂
1/26	6.7	0	6/6	5.0	0
2/2	5.0	0	6/8	6.7	0
			6/14	5.0	♂
			6/16	6.7	♂

*Signifies "not fertile."

13.7 days) and one other in which this period was 7.7 days. In two cases eggs were successfully fertilized 7.7 days after separation of the male.

The pair recorded in table 5 produced 34 eggs which were tested for fertility. Four eggs fertilized by normal sperm were all fertile. Also, all of the 11 eggs fertilized by sperm of 5 days or less of staleness were fertile. Four eggs, with 5.3 to 7-day periods were infertile. Two of the four tests made of a 7.7-day period were infertile.

The record given in table 6 was considerably influenced by the fact that most of the eggs laid were provided with inadequate shells. This and probably some other deficiency of the eggs doubtless prevented many of the embryos from completing their development. All of these eggs were fertile, and two of them were fertilized by very stale sperm (7.9 days). The male used in this instance was a pure-bred blond ring-dove. Since this was the only pure-bred successfully used in this study, and since the longest period tested was only 7.9 days and this proved fertile, we have no data on the question as to whether the sperm of pure-breds retains its fertility for a longer period than does the sperm of hybrids. This single instance affords some evidence that the fertility is not retained for a shorter time in the non-hybrid male.

TABLES 8 TO 10

Effects of fertilization with stale sperm in females B629 (top), A91 (center), and miscellaneous (below)

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
9/3	8.0	Some development	5/13	4.0	♂
10/19	(0)	(♀)	5/15	5.7	Soft-shelled egg
10/21	(0)	(6-day embryo)	5/21	4.0	5-day embryo
11/7	11.0	0*	5/23	5.7	5-day embryo
11/9	12.7	0	5/29	4.0	♂
12/11	(0)	(Some development?)	5/31	5.7	4-day embryo
12/20	4.3	Egg broken	6/6	4.0	6-day embryo
1/16	(0)	(?♀)	6/8	5.7	Egg disappeared
1/18	(0)	(3-day embryo, killed)	6/14	4.0	0
2/4	(0)	(7-day embryo)	6/16	5.7	0
2/6	(0)	(11-day embryo)[1]	6/22	4.3	0
2/15	7.0	0	6/30	5.0	0
2/24	5.0	0	7/2	6.7	0
2/26	6.7	Some devel.; broken	7/8	5.0	♀
3/5	5.0	Egg broken	7/10	6.7	0
3/14	4.0	♀	7/16	4.3	0
3/16	5.7	0	7/24	5.0	Slight development
3/23	4.1	0	7/26	6.7	0
3/31	4.0	♂	8/1	5.0	4-day embr.; killed
4/2	5.7	6-day embryo	8/3	6.7	Egg broken
4/8	4.0	4-day embryo	8/9	5.0	♂
4/10	5.7	5-day embryo	8/11	6.7	0
4/17	5.0	5-day embryo	8/17	5.0	Slight development[2]
4/25	4.0	♀	8/31	(0)	(Thin shell, broken)
4/27	5.7	3-day embryo; killed	9/2	(0)	(Soft-shelled egg)
5/5	6.0	Some devel.; broken	9/13	(0)	(♀)
5/7	7.7	Egg disappeared	9/24	(0)	(♀)
3/12	5.0	♂	4/22	4.0	3-day embr.; killed
3/14	6.7	♀	4/24	5.7	♀
3/21	5.0	Some development	4/30	5.0	7-day embryo[3]
3/23	6.7	♀	5/2	6.7	6-day embryo[3]
3/29	4.0	Trace of development	5/11	8.0	Egg disappeared
3/31	5.7	?♀; gonads atypical	5/13	9.7	Egg disappeared
4/6	3.2	0*	5/20	6.0	♀
4/8	4.9	9-day embryo	5/22	7.7	♀
4/14	5.0	♀	5/31	8.0	0
4/16	6.7	♂	6/2	9.7	0[4]

TABLES 8 TO 10—*Concluded*

♀ E562			♀ K363			♀ A60[5]		
10/27	12.0	0*	7/8	12.3	0	8/20	5.0	♀
1/8	15.0	0	7/10	14.0	0	8/22	6.7	♀
1/10	16.7	0	7/31	(0)	(♂)	10/10	(0)	(♂)
1/18	6.0	♀	8/2	(0)	(♂)	10/12	(0)	(♀)

* Signifies "not fertile."

[1] In this table, as in tables 3 and 6, it is probable that the early death of many of the embryos was causally associated with the abnormally thin shells of the eggs and with other deficiency within the egg.

[2] Male not copulating later; diseased, killed 8/24. Tubercular and with atrophied testes. Another male given 8/25.

[3] Death resulted from poor incubation.

[4] Female parent (A91) dead (internal hemorrhage, some tuberculosis) 28 days after.

[5] These three females refused in other trials to produce eggs when their mates were isolated.

The records for female 192 (table 7) and for the three additional females next to be considered are somewhat complicated by the fact that one or both of the parents became tubercular during the progress of the experiment. In these cases it is probable that part of the infertility found may be more properly assigned to disease in the parent than to the staleness of the sperm. The tests recorded in table 7 show much infertility, but this infertility is also shown in one of four tests with normal sperm. Further, the longer terms of staleness are not clearly associated with a higher percentage of infertility than are the shorter terms of staleness. The longest period tested was 7.7-days and this showed full fertility. Autopsy of the male at the close of the period studied showed tuberculosis and partial atrophy of the testes.

The tests recorded in table 8 are much like those found in table 7. In the present case, however, the absolute infertility of the two longest periods of staleness, 11 and 12.7 days, is probably significant. An 8-day period tested fertile although four of the five 6.7-day periods tested were wholly infertile. It will be noted that most of the fertile eggs of this record, like those of tables 3 and 5, produced embryos which died early.

Table 9 gives the result of 20 tests on fertility. All were fertile except the two tests made of the stalest sperm; the periods testing infertile were of 8 and 9.7 days. One test at 7.7 days and four at 6.7 days were fertile.

In table 10 is recorded the small amount of data obtained from three different females, (E562, K363, A60). Female E562 tested fertile in one case with sperm 6 days old, and three times infertile with sperm 12, 15 and 16.7 days old. Here the range of fertility apparently lies between 6 and 12 days. No. K363 was fully fertile in normal matings but was not fertile after a separation of the male for 12.3 and 14 days. No. A60 gave two fertile tests at 5 and 6.7 days. This female laid some soft-shelled eggs and others with shells which were probably too thin. Several of these eggs although of normal appearance, were fertile when fertilized by normal sperm but, like those already noted in tables 3, 5 and 8, most of the embryos died before hatching. All of the three females of this table failed to produce eggs when separated from the male except in the instances tabulated.

The record for fertility presented in table 11 is complicated not only by the fact that both parents were tubercular at the close of the experimental period, but by the additional circumstance that the male parent was a generic hybrid. Therefore, wholly apart from the matter of staleness of sperm and of the tuberculosis which developed in the parents, successful fertilization of all eggs could not be expected. Nevertheless the data seem to indicate that the staler sperm (5.7 and 6.7 days) are more frequently associated with infertility. None of the eggs laid longer than 5.7 days after separation of the parents was fertile although five such eggs were tested. Fertility in this pair of birds probably did not continue beyond 6 or 7 days. A notable summary is added to this table.

The summary on fertilization with normal and with stale sperm given in table 12 makes it clear that the stale sperm are associated with greater infertility than are the normal sperm. Also, the longer periods of staleness ("5 days," and "6+ days") show progressively greater infertility.

Stale sperm and the sex ratio. Whether in these experiments staleness of sperm affects the sex ratio may be next considered. The summarized data of table 12 can be first used to test this point. In this table only absolute infertility and embryos or offspring whose sex was learned are recorded. Embryos which died before their sex could be learned are left out of account. An adequate interpretation of the sex data requires a full and rather long discussion.

It will first be noted that we have not obtained a large number of male and female offspring from the "stale sperm fertilizations." The total number of such offspring is 213. Three divisions are made,

TABLE 11

Effects of fertilization with stale sperm in female A417

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
8/29	5.0	♂	4/7	4.0	♂
9/6	4.0	0*	4/9	5.7	0
9/8	5.7	0	4/15	4.0	♀ ; 10-day embryo
9/14	4.0	♂	4/17	5.7	0
9/16	5.7	0	4/23	4.0	Some development
9/22	4.0	?♀ ; gonads atypical	4/25	5.7	♂
9/24	5.7	Trace of development	5/1	5.0	♀
10/8	(0)	(2-day embryo)	5/3	6.7	0
10/10	(0)	(♂)	5/9	4.0	♂
10/17	5.0	0	5/11	5.7	0
11/4	14.0	0	5/17	4.0	♂
11/25	(0)	(2-day embr.; incub.?)	5/19	5.7	0
11/27	(0)	(0)	5/25	4.0	♂
12/21	(0)	(0)	5/27	5.7	0
12/23	(0)	(0)	6/11	(0)	(Some devel.; broken)
1/13	(0)	(♂)	6/13	(0)	(Sl. devel.; vasc. area)
1/15	(0)	(?♀ ; killed very young)	6/19	4.0	0
1/21	4.0	♂	6/21	5.7	0
1/23	5.7	Some development	6/27	4.0	0
2/16	(0)	(Trace of development)	6/29	5.7	0
2/18	(0)	(♀)	7/5	4.0	0
2/25	5.0	♂	7/7	5.7	0
2/27	6.7	0	7/13	4.0	♀
3/5	4.0	♂	7/22	5.0	♂
3/7	5.7	0; abnormal vasc. area	7/24	6.7	0
3/14	5.0	♂	7/30	4.0	0
3/16	6.7	0	8/1	5.7	0
3/22	3.0	4-day embr.; incub.(?)	8/7	4.0	Egg broken
3/24	4.7	Some devel.; incub.(?)	8/9[1]	5.7	0
3/30	4.0	♀			
4/1	5.7	2-day embr.; killed			
Total males and females from stale sperm fertilizations under this (left hand) column of tables 1 to 11 = 56♂♂ : 61 ♀♀.			Total males and females from stale sperm fertilizations under this (right hand) column of tables 1 to 11 = 39♂♂ : 54♀♀.		

*Signifies "not fertile."

[1] Both parents killed for autopsy 9/7; ♂A884 (generic hybrid) tubercular, with worms, and testes partly atrophied; ♀A417, tuberculosis and worms, ovary inactive.

however, of these data on the basis of the extent or degree of staleness. It will be seen that the total for all birds of the three divisions (table 12) shows an excess of females in all three divisions. This is also true for the

TABLE 12

Summary on the relation of stale sperm to fertility and sex

NUMBER OF FEMALE	STALE SPERM FERTILIZATION									NORMAL SPERM FERTILIZATION								
	2 to 4 days*			5 days*			6 and 6 + days*			During experiment			Immediately before experiment[1]			Previous year		
	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.
E361	8	3	0	9	8	1	8	11	5	4	1	0	6	10	0[2]	6	3	3[2]
49	3	9	2	2	5	4	0	4	10	4	9	0	6	7	4	40	44	3
B442	10	11	0	7	5	0	1	1	2	3	0	0	5	11	1			
A684	2	6	0	4	4	0	1	5	5	3	1	0	14	6	0	9	7	0
744	5	1	0	1	0	0				1	0	0	8	2	4			
79	1	5	0	5	5	3	2	2	2	2	1	0	6	13	1	6	7	1
Misc.				0	2	0	0	1	5	3	1	1	19	21	2	8	18	0
Total.....	29	35	2	28	29	8	12	24	29	20	13	1	64	70	12	69	79	7
192[3]	1	8	2	5	7	11	2	0	1	1	1	1	9	5	1	4	12	0
B629[3]	3	2	6	1	1	5	0	0	3	0	4	0	7	3	2	21	24	6
A91[3]	0	2	1	2	3	0	0	2	2				4	8	1	5	7	1
A417[3]	8	4	18[4]	4	1	4	0	0	1	2	2	3	6	8	4	16	16	1
Total[3].....	12	16	27	12	12	20	2	2	7	3	7	4	26	24	8	46	59	8
Grand total.	41	51	29	40	41	28	14	26	36	23	20	5	90	94	20	115	138	15

*All *pairs* of eggs in which the *first* of the pair falls within these limits are included. Also, periods to and including 4.9 are classed with 4-day periods; 5.9 with 5-day periods.

[1] The ten clutches immediately preceding the experiment are included.

[2] The male used in this period was not the same as that used during the experiment.

[3] One or both parents tubercular and this responsible for some of the infertility of the record.

[4] This large group (18) of infertile eggs is associated with the only *generic* hybrid male used in these studies; both parents tubercular at close of the experiment.

group of healthy birds of the three divisions if this group be separated from those birds in which disease developed during the experiment. Moreover, the excess of females is greatest (14♂♂ : 26♀♀) in the division pertaining to the stalest sperm. But does this mean that stale

sperms probably produce an excess of females? This question can be answered only by such control data as are added to this table and by an examination or analysis in which the conditions already known (Whitman (3); Riddle (4), (5), (6), and unpublished data) to affect the sex-ratio in doves and pigeons is considered.

TABLE 13

Showing number of males and females produced at precise periods of staleness and classified as to origin from specific order in pair or clutch

DAYS OF STALENESS	EGG OF CLUTCH			TOTAL
	First	Second	Single	
	♂♂ : ♀♀	♂♂ : ♀♀	♂♂ : ♀♀	♂♂ : ♀♀
0	12 : 6	11 : 12	2	23 : 20
2.2	1			0 : 1
3.3	1			0 : 1
3.9		1		0 : 1
4.0	21 : 21		1 : 3	22 : 24
4.3	1 : 2		1	2 : 2
4.4	1			0 : 1
5.0	21 : 20	1	2 : 2	24 : 23
5.2	1			0 : 1
5.3	2			0 : 2
5.7		15 : 19		15 : 19
6.0	9 : 12	1 : 1	3	10 : 16
6.1		1		0 : 1
6.3	1			1 : 0
6.7		15 : 15		15 : 15
6.9		1		1 : 0
7.0	2	1		1 : 2
7.7		4 : 9		4 : 9
Total for stale sperm.....	53 : 63	38 : 46	4 : 9	95 :118

Control data for each pair of birds used in the experiment are given under "normal sperm fertilization" in table 12. Three kinds or divisions of control data are available. These concern the sex ratio obtained from *a*, the previous year; *b*, the ten pairs or clutches laid immediately before beginning the experiment with stale sperm; and *c*, the

normal fertilizations which occurred during the experimental period. It will be observed that during the "previous year" (last column, table 12) more females than males were produced by these particular pairs of doves. More females than males were produced by both the healthy group and by the group of birds which became tubercular during the experiment (see footnote, table 12). Also the ten pairs of eggs which were laid "immediately before the experiment" (see next to last column, table 12) show an excess of females for the entire group of birds. The control data obtained from those fertilizations which were effected by normal sperm "during[3] the experiment" show, however, a slight excess of males (23♂♂ : 20♀♀).[4] It thus results that two divisions of the control data, like the three divisions of experimental data, gave an excess of females; one division of control data gave a contrary result. The excess of females (300♂♂:350♀♀) in these five divisions deserves a word of comment here. The slight excess of males (23♂♂ : 20♀♀) in the other division which was obtained from normal fertilizations within the experimental period also requires a further statement.

Whitman and Riddle have found that an excess of females is obtained when these doves are made to lay eggs during an extended period of time at an abnormally rapid rate. Tables 1 to 11 show that the stale sperm fertilizations which produced the sexes summarized in table 12 were in most cases fertilizations of eggs laid at an abnormally rapid rate,[5] and that this "crowded reproduction"—or reproductive overwork—was being continued for a period of at least many months. The complete data for the rate of egg laying during the previous year are not given here but the summary given in table 12 indicates that several of the pairs had been strongly "over-worked" during the previous year. As a matter of fact, all of the birds used for this study had previously been made to produce eggs very rapidly during ½ year to 4 years; in some cases, however, many or all of the eggs thus obtained were used for purposes other than incubation, and thus there are no sexes or few sexes recorded for the previous year. The fact of previous "overwork" also applies of course to the period designated "immediately

[3] For some of the pairs used in this study a small amount of data was obtained immediately after the close of the experiment; these data are included. In other words, all of the data of tables 1 to 11 are included.

[4] For the healthy pairs alone the excess is greater (20♂♂ : 13♀♀).

[5] Under normal conditions these doves would have produced probably not more than six to ten pairs of eggs per year.

before experiment." Therefore, the two last-named control periods and the three periods into which the "stale sperm fertilizations" are divided are all characterized as periods during which pairs of eggs were laid in rapid succession. All of these five divisions of the table show an excess of females.

On the other hand, the division which shows an excess of males, called "during the experiment" in table 12, includes practically all of those periods in which the females refused to produce eggs in the absence of the male. To this trial period, sometimes much prolonged, there was then usually added a period during which the male and female remained together before eggs were laid. Only 3 of the 43 offspring of this group (tables 1 to 11) were from eggs laid within 10 days or less after the previous pair of eggs. The remaining 40 were hatched from clutches with a time interval of 11 to 48 days, the average being 22.2 days. It is clear therefore that the division of table 12 which shows an excess of males includes the particular class of eggs which were *not produced at short time intervals*. In contrast with this group, the offspring of known sex which resulted from stale sperm fertilizations (first 3 divisions of table 12) were from eggs laid at quite short time intervals. The 148 clutches which produced such young (tables 1 to 11) have an average time interval of only 6.7 days with a range of variation of 5 to 17 days.

The above analysis of the data sufficiently shows that the sex ratio of the normal sperm fertilizations "during the experiment", and the different ratio found for stale sperm fertilizations, may be adequately accounted for on other grounds than of staleness or normality of sperm. The reason for the association of the *highest percentage* of females with the division of stalest sperm "6 and 6 + days" (table 12) can also be shown to be due to another factor than that of staleness of the sperm. The reason may be briefly stated as follows: The eggs which were fertilized by the stalest sperm were not distributed equally over the various portions of the year. In the early part of the study the parents were left together for longer periods after laying eggs than during the later part of the study; this resulted in a *shorter* interval between the "separation of the parents" (see second and fifth columns of tables 1 to 11) and the next succeeding ovulation in the earlier work. It was later realized that fertility was practically normal with sperm of 4, 5 and 6 days of staleness and that whatever additional data were obtained should preferably be concerned with longer periods of staleness. Such longer periods of staleness were secured by separating the male

from the female very soon after eggs were laid. But these prolonged periods of separation were thus largely concentrated into the *summer months* and after a *longer period of crowded or forced reproduction.* Reference to the right and left halves or divisions of tables 1 to 11 will demonstrate this point. Most of the longest periods of staleness (from which sex data were obtained) found there under the heading "days of separation of parents" will be found in the right-hand sections of the tables. The actual numbers (omitting table 10) are as follows: The left-hand halves of the several tables supply only 4 clutches or pairs of eggs whose sex is recorded in the "6 and 6+ days" column of table 12; while the right-hand halves of the tables supply 22 such clutches.

If one compares the sex ratios of all the stale sperm fertilizations of the right- and left-hand columns separately it will be found that the sum of the (earlier) left-hand columns shows a relatively higher percentage of males, —56♂♂ : 61♀♀; the right-hand columns total 39♂♂ : 54♀♀. As noted above, this later period (the right-hand columns) had been preceded by a longer period of reproductive overwork than had the earlier period and fell, for the most part, in summer.[6] In the work of Whitman and of Riddle to which reference has already been made this general situation was found to raise the proportion of female offspring. The group of offspring listed under "6 and 6+days" in table 12 arose therefore particularly from that portion of the year's record which experience has shown to yield a higher percentage of females, irrespective of staleness of sperm.

In the summaries given in table 12 the two eggs of a pair or clutch are recorded together, i.e., in the same interval column, although the intervals for the two eggs differ by 1.7 days. The results from each clutch have been thus recorded because earlier work has indicated that the females of pure species, and also of some hybrids to a less extent, more often throw males from the first egg of the pair or clutch and females from the second. In any comparison of sex ratios obtained from ring-doves it therefore seems more correct to carry the clutch as a unit. On the other hand, it is desirable to know the number of males and females produced at the actual or specific time intervals represented in the data, and thus make it possible better to judge whether there is a progressive increase of females (or of males) with increased staleness of the sperm. For this purpose table 13 has been constructed and a

[6] A still larger proportion of females than that obtained in summer may be had from the succeeding autumn, and perhaps even of the winter, if the females do not take a period of "reproductive rest."

summary separately presented for the two eggs of the clutch. If above-mentioned factors are considered the data indicate no such progressive increase. For example, it is true that the very longest period of staleness —7.7 days — gave 4♂♂ to 9♀♀; but these embryos are few in number, are from a "selected" group as already noted, and all are from the second egg of the clutch. Possibly the latter point has a bearing on the excess of females present, although table 13 makes it clear that the offspring of the "stale sperm fertilizations" as a group do not show different sex-values for the two eggs of the clutch.[7] The "normal sperm fertilizations," given in the top row of this table, do reflect the situation perhaps more commonly met with in these doves.

In connection with the topic last mentioned above attention should be called to a point in which the data of tables 1 to 11 are imperfect for a complete analysis of sex ratios. This imperfection arises from the fact that because of our wish to exceed the fertilizing period of stale sperm as often as possible, and because of special uncontrollable circumstances, sex has been ascertained in only about one-half (256 sexed, 218 not sexed) of the eggs produced during the period of the experiment. Also, unusually large numbers of the eggs obtained in this study disappeared or were broken, due to the activity of rats, handling, etc.; and in connection with these broken eggs is the circumstance that at least four of the thirteen females produced large numbers of eggs with inadequate shells. This frequently resulted in broken eggs and when the break occurred during incubation and escaped observation for a day or two the egg had to be recorded in tables 1 to 11 as "disappeared." Of still greater importance is the fact that these eggs with inadequate shells produced many embryos which died early—before sex could be ascertained.

We believe, however, that the imperfections of the data noted above still permit a fairly confident conclusion concerning the particular question of the relation of staleness of sperm to sex. The data obtained by us from ring-doves indicate that staleness of sperm had no appreciable or measurable effect on the sex ratios. The sex ratios obtained during the experiment cannot be considered normal, but the observed fluctuations can be accounted for in terms of significant factors previously investigated.

In the several preceding paragraphs the sex ratio obtained from stale sperm fertilizations—95♂♂ : 118♀♀—has been considered significant,

[7] The sex was obtained of fewer embryos from second eggs than from firsts (84 and 116 respectively), and more second eggs have inadequate shells (see tables.)

and as requiring consideration, not because these numbers are in themselves sufficiently large to establish their significance; but because much other work (Whitman (3) and Riddle, mostly unpublished data) has shown that much larger numbers, as well as considerably smaller ones, with evident regularity yield a similar excess of females under continuous and crowded reproduction.

DISCUSSION

Observations on the vitality of the spermatozoa of different animals demonstrate wide differences among the various classes and species in the length of time the sperm cells retain their fertilizing power. The time during which the spermatozoa retain their power of movement is also widely different for the various animal groups. Among the birds considered alone there are very considerable differences in the time during which the spermatozoa remain active in the female oviduct. The practice of some breeders of turkeys shows that these breeders confidently consider even a single union of the gobbler and turkey-hen sufficient to fertilize all eggs laid by the hen for a period of 3 or 4 weeks. The experiments of Lau (11) show that in the common fowl "some" eggs may be fully fertile for 19 days after removal of the male. After 19 days all eggs were infertile. Payne (12) reported no fertility after 16 days. Barfurth (13) quotes Harvey as stating that "infertility may be calculated to continue twenty to thirty days after separating hens from cocks." The Ontario Agricultural College Report for 1898 records a test in which all eggs were infertile after the 9th day. Concerning the time during which sperm cells of the fowl remain motile there is even wider discrepancy in the observations. Payne reports motile sperm in the oviduct at 56 days, although the number of sperm cells was much reduced after 14 days. Barfurth found no live cells in the fowl's oviduct at a 22- to 24-day period. Lau found active sperm in the sperm ducts of males 24 days after castration.

The results described in the present paper indicate that the spermatozoa of most of the ring-doves studied by us retain their fertilizing power during very close to 8 days. For the pair described in table 11 the time is almost certainly less than 7 days, but the use of the sperm of a generic hybrid male is possibly the explanation of the reduced period in this case. In all of the tests made with the several pairs of doves two eggs only tested fertile at 8 days. Three additional tests at 8 days, one test at 8.7 days, eleven tests at 9 to 13.7 days, and four tests at 14 to 17 days all proved infertile. In all of the above instances the

period of staleness is calculated from the time of separation of the male to the hour at which the egg is laid. It is almost certain that if the egg is fertilized at all this must occur at least 24 hours before laying. All of the figures for staleness of sperm given by us would therefore be made more accurate if one day were deducted from the period as stated.

It is possible that the one notable variation from the usual period of fertility among the pairs of ring-doves studied by us is an individual difference and is not necessarily associated with the hybridity (two genera) of the male parent. Such individual differences probably exist in fowls. Otherwise it is difficult to explain the results obtained on the limits of fertility in the fowl as this has been reported by various authors and cited above.

Only one male of a pure species was successfully tested in the present study. The tests made upon the sperm of this male all showed full fertility but the tests did not exceed a 7.7-day period. Four other attempts to test similar males were unsuccessful. Whether the sperm of males of pure species retains its fertilizing power for a longer period than does the sperm of hybrids is a question which remains undecided. Also, no attempt has been made to learn the length of time during which the spermatozoa of these doves remain motile in the female oviduct. At present we know very little concerning the question whether the sperm of one species of doves will live longer in the oviduct of a bird of the same species than in that of a different species or genus. The results reported in the present paper are, however, practically unaffected by differences possibly involved in that question.

In the introductory paragraphs of this paper reference has already been made to a possible or conceivable relation of stale sperm to abnormal sex ratios. Although staleness or "over-ripeness" of the ova of the frog was one of the observed facts associated with the production of the extremely high percentages of males obtained by Hertwig and by Kuschakewitsch, it has been made fairly clear by King's (14) further study on the toad that the change observed in the sex ratio is possibly associated with a changed moisture-content which occurs during the "over-ripening" of the egg, and not necessarily with any value that may be assigned to "staleness" *per se*. It has been earlier pointed out (6), (15) that the ova of all vertebrates thus far studied in this respect take up moisture from the oviducal or uterine fluids which they meet after extrusion from the ovary. And further, that this change in the state of hydration of the egg affords a possible basis for the interpretation of the abnormal sex ratios thus obtained, since the male and

female-producing ova of doves and pigeons show similar differences of hydration (together with other differences) according to their prospective sex value.

The available evidence therefore gives some reason for attributing a sex-modifying value to staleness of ova which is thus far wholly lacking in the case of the sperm. The positive result[8] of the tests made on the (indirect?) effects of staleness of ova on the sex ratio in the frog (Hertwig, Kuschakewitsch) and the negative tests which we have now obtained on the spermatozoa of pigeons conform to the above-stated expectation. In other words, it was known that staleness of ova favors a change (increase) in the moisture-content of the ovum. Hertwig, Kuschakewitsch and King changed the moisture content of the ova in frogs and toads and apparently obtained abnormal sex ratios; but spermatozoa have not thus far been shown to undergo any definite change in their moisture-content (although this may be wholly due to the great difficulties of making the tests) during their continued lodgment in the female genital tract, and a test of the effect of such delay upon the sex ratio in the case of doves has shown the absence of any measurable effect.

The results of the 213 successful individual tests made by us with stale spermatozoa of ring-doves show no appreciable or measurable effect on the sex-ratio. In conclusion, we would point out that this fact has a further and wider application, since it is now clear that the abnormal sex ratios that have been previously obtained by investigations (Whitman, Riddle) on such doves are not complicated by nor causally associated with staleness of spermatozoa.

SUMMARY

The spermatozoa of the ring-doves used in this investigation retained their fertilizing power during nearly 8 days. This period represents the interval between the hour of isolation of the male and the hour the egg is laid.

Variations in the period of staleness compatible with fertilizing power in various bird species and in individuals of the same species are discussed.

[8] Swingle, (Amer. Nat., liv, 1920) has very recently questioned the validity of the cytological criteria used by previous workers in classifying male and female Anuran larvae. Still more recently, however, Crew (Proc. Roy. Phys. Soc. of Edinburg, xx, 1921) has obtained new evidence of at least a considerable amount of true hermaphroditism in the frog.

No evidence was obtained indicating weakness or modified viability in the embryos obtained from stale sperm fertilizations. Some of the parents used in this study, however, produced numerous eggs within which the embryos were unable to complete their development. The cause of these failures seems to be associated with inadequate egg-envelopes and with deficiences of the ovum which have been made the subjects of further investigation.

Staleness of the spermatozoa did not appreciably affect the sex ratio in the birds studied. Some of the sex ratios obtained during the experiment cannot be considered normal but these abnormal ratios have been shown to be associated with other factors investigated earlier.

The abnormal sex ratios that have been obtained in previously reported investigations on these doves, and any results that may be later obtained from them or from similar birds, are here shown to be probably not measurably complicated by effects due to staleness of the spermatozoa.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

X. Inadequate Egg Shells and the Early Death of Embryos in the Egg

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Received for publication June 6, 1921

Some doves and pigeons have been found to produce extraordinarily large numbers of eggs within which the embryos die before hatching. Some of these birds also occasionally produce a fairly continuous series of embryos which die at progressively earlier stages in the later eggs of the series. Doves which yield such series of dead embryos or which produce large numbers of non-viable embryos have been found to enclose some of these in soft shells or in demonstrably thin or inadequate shells and still others of these early-dying embryos in quite normal shells. The occasional egg with soft shell or with inadequate shell therefore becomes a sign of some more deeply seated defect or trouble. The main purpose of this paper is to show that the abnormally high numbers of dead embryos and the inadequate shells are thus loosely yet actually associated.

The present problem is of course a part of the general problem of the causes of death in fetus and embryo. It has a further special bearing upon the enormous annual economic losses which occur from failures of this kind in the poultry industry since it is well known that of the total number of eggs incubated in any country there is a high percentage of eggs either infertile or within which the embryos die before hatching. Probably this is the principal source of avoidable loss in that industry. A part of this infertility and embryonic death is doubtless wholly unrelated to anything in any way associated with inadequate egg shells; but that such egg shells have hitherto undemonstrated associations with these phenomena, and that these latter are utilizable in the poultry industry and elsewhere in reducing the losses resulting from the incubation of unhatchable eggs, will be indicated in the present paper.

In the preceding study of this series it was found by Riddle and Behre (1) that certain ring-dove females produced eggs in which the embryos usually died after a few days of development. Most of these same females were known to be producing some soft-shelled eggs or eggs obviously thin-shelled and easily broken. From this fact it may be inferred that the death of many of the sister-embryos enclosed in intact and apparently good shells was somehow related either to an inadequacy of their own egg-envelopes or to the production by the same parent of occasional thin or inadequate shells.

Since the high proportion of dead embryos noted above occurred in an experiment dealing with the effects of "stale" spermatozoa, and since the dead embryos found there were in greater numbers than have hitherto been observed by us, it seems advisable to supply at once to those not familiar with this material sufficient evidence that there is no necessary or real connection between the staleness of the sperm used and the extraordinarily high death-rate of so many young embryos. Moreover, the sex studies being conducted by the author require the development of all embryos to at least a stage at which their sex may be learned; the cause of failure to reach such a stage is a point of real significance in those studies. In this previous work on sex and fertility there have been encountered phenomena of partial fertility or of restricted life-terms, which it is quite necessary and possible to distinguish from death of embryos due to extrinsic causes or to disease or nutritional disorder in the parent. Much completed work and other studies now in progress have therefore made necessary a search for the real cause of the death of embryos contained in, or in any way associated with, egg shells which are abnormal or inadequate. The statements just made give our own special reasons for a study of this problem.

Presentation of data. The investigation dealing with stale sperm (1) is published simultaneously with the present study and the tabulated data of that paper may be consulted with reference to the production by certain birds of series of eggs within which the embryos died early; also the opportunity is offered there to note the tendency toward shorter life-terms from later or succeeding eggs produced by a particular bird. It can there be observed also that birds which at the end of the record were producing many non-viable embryos had earlier produced large numbers of wholly normal and healthy embryos.

Soon after the conclusion of the above-mentioned study we began an examination of the adequacy of the shells of all of the then-living

TABLE 1

Loss of weight of eggs from different female ring-dove hybrids. In an earlier study (1) few embryos died in eggs of females grouped in top rows; many died in eggs of females grouped in bottom rows; an intermediate number in middle rows

NUMBER OF FEMALE		DATE OF EGG	WEIGHT OF EGG	AVERAGE	LOSS IN MILLIGRAMS PER HOUR AS SHOWN BY SUCCESSIVE WEIGHINGS. NUMBER OF HOURS IN PARENTHESES						Average
					Early		Later		End		
			grams								
Group 1	E361	9/16	9.1				(240)	3.9	(314)	3.9	
		9/23	8.5		(24)	3.3	(26)	3.8	(324)	3.7	
		9/25	9.3		(26)	4.0	(22)	4.8	(310)	4.2	
		10/4	8.0						(336)	3.6	
		10/6	8.3						(293)	6.3	
	A684	9/13	8.4						(335)	4.0	
		9/15	8.8						(308)	4.1	
		9/22	8.7		(19)	3.0	(24)	3.9	(330)	3.5	
		9/24	9.4		(24)	3.9	(26)	4.6	(320)	4.6	
		10/5	8.3						(330)	3.6	
		10/7	8.6	*8.7*					(312)	3.4	*4.1*
Group 2	49	9/16	9.1						(336)	3.7	
		9/18	9.4						(314)	4.5	
		9/25	9.2		(24)	2.8	(24)	3.1	(328)	3.6	
		9/27	7.5		(24)	3.6	(24)	4.2	(308)	4.6	
	79	9/15	7.4						(309)	3.6	
		9/25	8.0		(24)	3.7	(24)	3.1	(327)	4.0	
		9/27	7.1		(24)	3.4	(24)	3.7	(309)	3.8	
		10/8	7.4						(333)	3.4	
		10/10	7.6						(291)	3.5*	
	192	9/12	8.7						(337)	3.7	
		9/14	8.6						(295)	4.7	
		9/20	8.1		(19)	4.0	(24)	3.5	(331)	3.3	
		9/22	9.0		(19)	6.8	(24)	6.3	(312)	5.8	
		10/8	9.2		(148)	4.9			(148)	4.9*	
		10/14	8.0		(48)	2.8	(48)	5.6	(96)	4.2[1]	
		10/16	8.9	*8.3*	(7)	4.8	(48)	5.3	(55)	5.2[1]	*4.2*
Group 3	744	10/5	9.2		(24)	3.3	(25)	2.3[2]	(234)	2.7*	
		10/7	9.2		(24)	4.3	(24)	4.5	(312)	3.9	
		10/14	9.2		(25)	2.8	(46)	3.3	(119)	2.9*	
		10/16	8.9		(25)	7.4	(46)	7.1	(78)	7.2*	

TABLE 1—*Concluded*

NUMBER OF FEMALE		DATE OF EGG	WEIGHT OF EGG	AVERAGE	LOSS IN MILLIGRAMS PER HOUR AS SHOWN BY SUCCESSIVE WEIGHINGS. NUMBER OF HOURS IN PARENTHESES						Average
					Early		Later		End		
			grams								
Group 3	B442	9/22	8.3		(19)	3.0	(26)	1.0[3]	(306)[4]	3.1	
		9/24	8.2		(26)	1.6[3]	(24)	5.0	(192)[4]	5.2*	
		10/1	8.2		(23)	2.7	(23)	2.7	(211)	2.8*	
		10/3	8.1		(23)	5.2	(22)	4.5	(319)	4.8	
		10/10	8.2		(8)	2.9	(24)	3.4	(219)	3.1*	
		10/12	7.3		(24)	5.1	(20)	5.0	(320)	5.0	
	B629	9/13	8.6						(273)	5.1	
		9/24	8.6		(26)	6.5	(22)	5.5	(304)	5.8	
		10/5	9.0		(25)	3.6	(24)	4.8	(230)	5.3*	
		10/7	9.5		(24)	11.5	(25)	10.8	(312)	10.1*	
	A60	9/24	8.8		(22)	4.6	(27)	5.0	(311)	4.6	
		9/26	9.3		(22)	9.3	(27)	9.4	(310)	8.1	
		11/26	9.5		(189)	4.5	(68)	6.2	(306)	4.3	
		11/28	10.2	*8.8*	(147)	9.7			(147)	9.7[1]	*5.2*

* Produced embryos which died before hatching. All other eggs hatched.

[1] Embryos killed at early age in an experiment (where they were placed by mistake); the possibility of their living to end of incubation not tested.

[2] Shell reinforced with tape which lessened rate of loss.

[3] Egg left at room temperature (not incubated) during this period.

[4] Exclusive of time kept at room temperature.

birds used in the earlier study. Much earlier experience with soft-shelled and thin-shelled eggs had shown that those eggs with least shell material lost weight most rapidly. For example, an egg that had a very thin deposit over one-half its surface, and shell membrane only over the other half, showed a rate of loss at room temperature of 0.121 to 0.145 gram per hour for 17 hours. This was 84 times the rate at which an egg with presumably normal shell lost weight under the same conditions. Practically all intermediate rates of loss have been found and measured and it seems that, without special consideration of such data, we may here treat relative rates of loss from eggs as a measure of their relative permeability to water or water vapor. During the first day after the egg is laid the loss is in fact essentially nothing else than a loss of water; though later there is of course a perceptible loss through CO_2-production and a gain through

O_2-fixation; but these latter tend to balance each other, and the unsatisfied balance is essentially equalized between different eggs by the circumstance that embryos are present in all of the eggs compared. Eggs containing embryos dying prematurely were weighed and opened very soon after the death of the embryo so that rates of loss as measured indicate the rate while a living embryo was present. This rate is reduced after the death of the enclosed embryo. For most of the embryos the rate of loss was obtained at five successive periods; the rates obtained for three of these periods are found in table 1.

An examination of tables 1 to 11 of the earlier paper (1) will show that those females which we have here grouped at the top of table 1 had produced no eggs with soft shells; none of their eggs were later broken, and the eggs laid by them showed the smallest proportion of dead embryos. Similarly those females placed in group 2 of table 1 will be found to have shown a slightly greater proportion of these abnormalities. Group 3 of the table includes those females which in the earlier investigation produced relatively the greatest number of soft-shelled eggs, broken eggs and dead embryos.

A comparison of the average egg weights and average rates of loss of water by the three above-mentioned groups makes it clear that the group which produced most dead embryos—in both the earlier and the present study—also has the highest rate of loss[1] of water (5. 2 mgm. per hour); and the group with lowest rate of loss (4.1 mgm. per hour; egg weight = 8.67 grams) corresponds to the group that produced—in both the earlier and the present study—fewest dead embryos.

If one looks for evidence of a high rate of loss associated with the death of specific or individual embryos within group 3 of table 1 the data are confusing and quite inconclusive. Other factors than rate of loss are plainly involved. All individual eggs with high rate of loss do not die and all individual eggs with lower rates of loss do not survive and hatch. It is clear only that the *group* of birds which experience had

[1] The actual rate of weight-loss from the egg is of course much influenced by the temperature to which it is exposed as well as by the thickness of shell. In order to show this the first two eggs of ♀ B442, shown in table 1, were left during 26 hours at room temperature (about 20°C.) with the result that the loss was reduced to about one-third normal. All doves incubate inconstantly until the second egg of the pair is laid. Of course the rate of loss is related to the amount of shell surface; the latter, however, can be measured only with difficulty and since surface bears a close relation to weight, the weight of each individual egg is given in the tables.

shown to produce occasional soft-shelled eggs and many dead embryos in apparently good shells is the group of birds whose eggs have the thinnest shells. Also that at least some of the birds which produce thin shells occasionally yield shells which are thicker than is normal; this fact emphasizes the disordered condition of these oviducts.

A few months later a second lot of data was collected. At that time there were six birds in our collection which had recently produced one or more soft-shelled eggs. These six birds were taken for careful study (table 2) and three females producing only eggs with presumably normal shells were taken as control (table 3). All eggs produced by these nine birds during a period of 5 weeks were supplied with the most careful incubation under the most trustworthy birds; their rates of loss were twice determined and the age attained by their enclosed embryos recorded. The data of table 2 contrast with those of the control (table 3) in quite the same way that group 3 of table 1 has been seen to contrast with group 1 of that table. All of the six birds of table 2 gave one or more dead embryos, and the lowest average rate of loss from a bird of this group was higher than the highest average for the control.

During the year that has elapsed since the above data were obtained many data have been obtained from time to time. All of the latter data have very fully confirmed those given in tables 1 to 3. The normal rate of loss for eggs of these ring-doves lies between 3.0 and 4.0 mgm. per hour. Birds which produce soft-shelled or obviously thin-shelled eggs produce also many others with apparently normal shells whose rate of loss exceeds 6.0 mgm. per hour; and an unmistakably high proportion of the embryos die before hatching. Here also the dead embryos are found from eggs of low as well as of high rate of loss. The tabulation of these records would require considerable space and would add nothing except volume to the better-controlled data already presented.

One further fact concerning the origin of inadequate egg shells is made clear by the data, namely, that the inadequate shells are much more often found on the second egg of a pair than on the first. The eggs are laid 2 days (40 hours) apart and to make the above comparison possible the date of laying is given in tables 1 to 3. A summary of this situation is given in table 4, where it can be seen that 47 first eggs of the pair or clutch had an average loss of 3.7 mgm.; 46 second eggs an average loss of 5.7 mgm. A similar difference is found in all

TABLE 2

Rate of loss from eggs and survival of embryos from all eggs laid during a period of five weeks by all ring-dove females known to be producing some eggs with thin shells. All eggs incubated under birds

NUMBER OF FEMALE	DATA ON EGGS		RATE OF LOSS (IN MILLIGRAMS) PER HOUR (5-DAY PERIODS)			STAGE ATTAINED BY EMBRYO
	Date laid	Weight	First period	Second period	Average	
B629	4/5	8.4	8.5*	8.0	8.3	Hatched
	4/22	7.5	5.4	4.6	5.0	Hatched
	4/24	8.5	9.1	7.8	8.5	Dead, 11-day embryo
	5/1	8.2	5.6	4.6	5.1	Hatched
	5/3	8.5	11.6*	9.3	10.5*	(Dead?)[1] 11-day embryo[2]
	Av.	*8.2*	*8.0*	*6.9*	*7.5*	
B442	4/6	7.9	5.0		5.0	Dead, 3.5-day embryo
	4/15	8.3	2.6	2.5	2.6	Dead, 7-day embryo
	4/17	8.5	5.7	5.7	5.7	Hatched
	4/23	7.7	3.0		3.0	Dead, 4.5-day embryo
	4/25	8.4	6.6		6.6	Dead, 3-day embryo
	5/2	8.3	2.8		2.8	Dead, 1-day embryo
	5/4	8.5	6.4		6.4	Dead, 1-day embryo
	Av.	*8.2*	*4.6*	*4.1*	*4.6*	
K465	3/31	8.5	4.6	4.4	4.5	Hatched
	4/2	8.7	6.6	5.6	6.1	Hatched
	4/12	8.5	3.5	3.2	3.4	Hatched
	4/14	8.6	5.2	4.6	4.7	Hatched
	4/21	7.8	3.8	2.6	3.2	Dead, 13-day embryo
	4/23	Soft shell			(?)	
	4/30	6.9	3.8	3.6	3.7	Hatched
	5/1	8.2	8.1		8.1	Dead, 2.5-day embryo
	Av.	*8.2*	*5.1*	*4.0*	*4.8*	
A60	4/15	8.1	3.5	4.3	3.9	Dead, 14-day embryo
	4/17	9.3	5.4	5.6	5.5	Hatched
	4/24	8.7	3.9	4.5	4.2	Hatched
	4/26	10.1	6.9	7.5	7.2	Hatched
	5/4	8.9	4.0	4.5	4.3	Hatched
	5/6	10.2	6.3	6.4	6.4	Hatched
	Av.	*9.2*	*5.0*	*5.5*	*5.3*	
P431	4/4	8.7	3.8	4.2*	4.0	Hatched
	4/6	9.1	9.6*		9.6*	Live (?), 6.5-day embryo[2]
	5/1	8.5	3.9	3.4	3.7	Dead, 13.5-day embryo
	5/3	9.4	7.1	6.2	6.7	Hatched
	Av.	*8.9*	*6.1*	*4.6*	*6.0*	
K954	4/11	9.1	8.9	8.8	8.9	Dead, 5.5-day embryo
	5/4	9.2	4.5		4.5	Dead, 2.5-day embryo
	5/6	10.0	(27.5)*		(?)	Live, 4-day embryo[2]
	Av.	*9.4*	*6.7*	*8.8*	*6.7*	
	Average for group = 5.6 mgm. per hour					

* Egg slightly broken; repaired with tape.

[1] It is possible that a further slight break in shell on 5/14 was cause of death.

[2] Egg broken.

of the five groups which are included in this average.[2] It is also true that eggs from birds which are laying presumably only normal shells show

TABLE 3

Rate of loss from eggs and survival of embryos from all eggs laid during a period of 5 weeks by females thought to be producing eggs with normal shells (control data for table 2)

NUMBER OF FEMALE	DATA ON EGGS		RATE OF LOSS (IN MILLIGRAMS) PER HOUR (4–7 DAY PERIODS)			STAGE ATTAINED BY EMBRYO
	Date laid	Weight	First period	Second period	Average	
P583....	3/31	9.0	4.0	4.2	4.1	Hatched
	4/2	9.4	5.2	5.5	5.4	Hatched
	4/10	8.0		3.3	3.3	Hatched
	4/12	9.0		4.6	4.6	Hatched
	4/18	8.8	3.5	4.0	3.7	Hatched
	4/20	9.4	4.5	4.2	4.3	Hatched
	4/26	8.6	3.2	3.5	3.3	Hatched
	4/28	9.5	4.5	5.1	4.7	Hatched
	Av.	*9.0*	*4.2*	*4.3*	*4.2*	
P728....	3/31	8.6	2.9	3.2	3.0	Killed or died at 12 days
	4/2	9.2	3.2	4.0	3.6	Killed or died at 10 days
	4/10	8.3		3.1	3.1	Hatched
	4/12	9.0		3.4	3.4	Hatched
	4/18	9.2	5.0	5.2	5.1	Hatched
	4/20	9.6	4.8	5.0	4.9	Hatched
	4/26	9.2	3.3	3.8	3.5	Hatched
	4/28	10.1	3.6	5.5	4.5	Hatched
	Av.	*9.2*	*3.8*	*4.2*	*3.9*	
P720....	3/29	6.8	2.8	2.9	2.8	Hatched
	3/31	7.1	3.9	3.8	3.9	Hatched
	4/11	7.3	2.5	2.7	2.6	Hatched
	4/13	7.4	4.0	4.0	4.0	Hatched
	4/20	6.6	3.0	3.1	3.0	Hatched
	4/22	7.6	4.2	4.3	4.2	Hatched
	4/29	6.5	3.8		3.8	Dead 5-day embryo
	5/1	7.3	6.3		6.3	Hatched
	Av.	*7.1*	*3.8*	*3.5*	*3.8*	
	Average for group = 4.0 mgm. per hour					

[2] The second egg of the pair is usually, but not always, larger than the first. With a slightly greater surface area it should of course show a proportionately higher rate of loss. An inspection of the data obtained from birds which produce thin-shelled eggs clearly shows, however, that the second eggs have disproportionately high rates of loss.

much less difference between the shells of the two eggs of the pair than do the eggs of birds which are known to be producing some thin-shelled or soft-shelled eggs. Thus the first eggs of twelve pairs in table 3 show a rate of loss of 3.4 mgm. per hour and the seconds 4.6 per hour; and sixteen pairs from table 2 have an average loss of 3.9 mgm. per hour for firsts while the seconds show a loss of 7. 1 mgm. per hour. The data of the tables make it clear, however, that some first eggs, and also some single or unpaired eggs, are provided with inadequate shells, and that embryos die in all classes of eggs.

As measured by relative rates of loss the second egg of the pair has been shown to have the thinner shell. The data collected in table 5 show that the second eggs also contain less than their proper proportion of dry shell material. The table includes all of the control[3] data

TABLE 4

Showing that the second egg of the pair or clutch usually has a less adequate shell (as measured by rate of loss of weight by egg) than the first of the pair

SOURCE OF DATA	FIRST OF PAIR			SECOND OF PAIR		
	Number of eggs	Weight of eggs	Rate of loss	Number of eggs	Weight of eggs	Rate of loss
Group 1, table 1.....	5	8.4	3.7	5	8.9	4.5
Group 2, table 1.....	7	8.4	3.7	7	8.3	4.6
Group 3, table 1.....	8	8.8	3.6	8	8.8	6.8
Table 2.....	15	8.3	3.9	14	8.4	7.1
Table 3.....	12	8.1	3.4	12	8.7	4.5
Average (weighted).....		8.4	3.7		8.6	5.7

obtained in the two next following investigations of this series of papers (2), (3), and are given here because the condensed tabulations of those papers prevent their inclusion there. In only one pair of eggs shown in table 5 was the second egg smaller than the first and in three pairs the two eggs were of equal weight; but the data show thirteen pairs in which there was absolutely less shell material on second eggs than on the corresponding firsts. Fewer figures are at hand for the weight of ash and these are less indicative of a difference than are the figures for dry shell material. The percentage of ash in the dry shell material seems to favor neither first nor second egg of the pair. The more com-

[3] Three birds either ceased producing eggs or began producing abnormal eggs (see Riddle and King (3) table 3) soon after dosage with atropine. The "control" eggs obtained in this latter period are here excluded.

Showing amounts and relative amounts of shell material found on the first and second eggs of ten normal ring-doves. (The eggs are all in pairs; the first of the clutch is written first)

WEIGHT IN GRAMS			PERCENTAGE OF		WEIGHT IN GRAMS		BASES (AS $CaCO_3$) IN ASH	PERCENTAGE OF SHELL IN EGG WEIGHT
Egg	Shell (dry)	Ash	Shell in egg weight	Ash in Shell	Egg	Shell (dry)		
8.2	0.4388	0.2348	5.349	0.5351	9.5	0.4473	94.95*	4.719
9.2	0.4655	0.2422	5.061	0.5202	9.3	0.4255	95.43	4.583
8.4	0.4374	0.2346	5.211	0.5364	9.6	0.4528	95.67	4.711
8.9	0.4405	0.2343	4.926	0.5319	10.4	0.4969	95.55	4.761
7.0	0.3311	94.69*	4.734		9.3	0.4388	95.66	4.730
7.1	0.3755	95.76	5.268		10.4	0.4788	95.77	4.584
9.4	0.4758		5.070		8.2	0.4031	95.17	4.888
10.8	0.5067		4.705		8.8	0.4324	96.22	4.909
9.5	0.4599	0.2470	4.816	0.5372	9.2	0.4619	95.55	5.046
11.3	0.4655	0.2513	4.132	0.5398	9.5	0.4631	95.83	4.892
9.2	0.4287	95.83*	4.653					
10.3	0.4429	95.60	4.316		8.4	0.4689		5.584
					9.3	0.4497		4.848
9.6	0.4523	95.77	4.711		8.3	0.4558		5.517
10.6	0.4686	95.92	4.403		8.8	0.4166*		4.750
8.8	0.4356	95.17	4.932		8.6	0.4361	95.51	5.082
10.6	0.4362	95.49	4.117		8.8	0.3918	95.13	4.472
9.7	0.4635	93.86	4.759					
10.5	0.3891	95.32	3.723		8.8	0.4285	95.81	4.885
					9.4	0.4813	95.40	5.115
7.0	0.3440	0.1856	4.936	0.5395	8.6	0.4644	95.81	5.427
8.1	0.3619	0.1925	4.452	0.5319	9.2	0.4170	95.59	4.532
					8.4	0.4513	95.30	5.354
7.9	0.3704[1]	0.1891	4.705	0.5105	8.9	0.4206	96.20	4.740
8.9	0.3930[1]	0.2054	4.412	0.5226				
8.0	0.3733	0.1910	4.694	0.5117	8.9	0.4183		4.700
8.9	0.3854[1]	0.2045	4.336	0.5305	9.0	0.4370		4.858
					8.6	0.4114	95.75	4.798
7.9	0.3995	0.2157	5.049	0.5399	8.6	0.4243	95.88	4.945
8.6	0.4245	0.2271	4.928	0.5350				
8.0	0.4034	0.2160	5.035	0.5354	8.8	0.4302		4.883
8.8	0.4252		4.853		9.3	0.4165		4.486
7.5	0.3420	95.54*	4.559		8.4	0.3959		4.725
8.3	0.3860	95.71	4.660		9.0	0.3981[1]		4.413
					8.7	0.4148	96.28	4.785
8.0	0.3752	95.83	4.695		8.9	0.4130	95.74	4.634
8.7	0.4010	95.93	4.627					
7.8	0.3445	94.30	4.404					
8.3	0.3700	95.33	4.451		8.1	0.3904	96.12	4.832
					9.1	0.4141	95.77	4.543
7.0	0.3171	95.84	4.518		8.9	0.3879	95.64	4.363
8.2	0.3604	96.09	4.603		9.3	0.3780	95.47	4.057
7.7	0.3515	96.20	4.558		8.9	0.3692	95.70	4.126
8.3	0.3566	96.29	4.310		8.9	0.3583	95.33	4.031
8.8	0.4510	0.2299	5.135	0.5097	8.3	0.4542		5.489
9.5	0.4778[1]	0.2538	5.021	0.5313	8.3	0.4506		5.421
8.6	0.4477	0.2412	5.183	0.5388	7.7	0.4277	96.11	5.565
9.3	0.4799	0.2603	5.148	0.5425	8.1	0.4562	95.68	5.639

* Percentage of bases calculated as $CaCO_3$ in shell.

[1] Traces of shell membrane present.

plete comparison of the percentages of alkaline bases (calculated as as $CaCO_3$) for the two eggs of the clutch indicates that the ash of the second egg contains quite as much of these bases as is found in the ash of firsts; the relative amount of shell materials from which these figures were derived seems also nearly normal. Twenty-five first eggs with an average weight of 8.5 grams had 0.4091 gram of shell, of which 95.52 per cent was $CaCO_3$; while the same number of second eggs had an average weight of 9.1 grams with 0.4175 gram of shells, of which 95.70 per cent was $CaCO_3$.

The numerous figures for the percentage of total weight which is represented by shell requires detailed examination, as well as a summary such as is given below, in order to discover a tendency on the part of second eggs of some of the birds to show significantly[4] lower proportions of shell material. The 42 first eggs of the table have an average weight of 8.5 grams; their dry shells a weight of 0.4155 gram; and their shells average 4.903 per cent of the total weight. The 42 second eggs have an average weight of 9.2 grams, their dry shells average 0.4246 gram, and their shells average 4.658 per cent of the total egg weight. This definitely lower percentage of the total egg weight represented by the shells of the second eggs is probably significant. The several instances noted above in which the smaller first egg of the pair received absolutely more shell than its associated second are certainly significant. Altogether these results are of additional value because all of the eggs concerned were derived from birds which produced only eggs with presumably normal shells—they certainly produced no obviously thin-shelled eggs.

DISCUSSION

Our search for the cause of the obscure defects and disturbances here described naturally led us to inquire whether the defect is a matter of inheritance. Among the ring-doves, which have been the principal material studied by us, we do find a relatively large number of affected birds among the descendants of a particular ancestral pair of doves; but the disturbance has also been found in offspring of various and widely different ancestry. The trouble may arise, moreover, in the later eggs of an individual bird which has produced many and

[4] Since the second eggs are slightly larger their shells may be of equal thickness with those of first eggs and yet show a *slightly* lower proportion of shell weight to egg weight.

only apparently normal shells and practically only viable embryos during one, two or more earlier years of her life. This striking fact is opposed to the assumption that lethal factors in particular or heredity in general are largely, if at all, involved. Nearly all of the available data indicate that the disorder has a temporary and physiological basis.

The disturbance has been observed in many kinds of hybrids and in pure species of several different genera and families of pigeons. Among these latter are Columba (2 species), Turtur, Spilopelia, Stigmatopelia and Zenaidura. It is well known that soft and inadequate shells occur in the common fowl and although we have not made a special study of the early-dying embryos of that bird it may be safely assumed that conditions there are in the main similar to those among pigeons. Among poultrymen it is rather generally assumed that an egg with a soft or thin shell merely signifies that the egg was expelled prematurely from the oviduct. That this premature expulsion of the egg does sometimes occur is unquestionable for both fowls and pigeons. In pigeons, however, it is certainly exceedingly rare. Furthermore, in pigeons, the definite hour or time of egg-laying has permitted us to know positively in these cases that the egg was in the oviduct during exactly the normal number of hours.

The special deficiency of the shells of second eggs of the pair as described above would seem to indicate that the secretion of an adequate shell is in some way facilitated by the inactivity of the shell gland during 6 or more days prior to the laying of the first egg. The secretion of the second shell follows soon after the conclusion of the shell secretion for the first—within 10 to 15 hours—and in each case the gland is active during about 30 hours. In other words, the data suggest that the bird's store of shell-forming materials is depleted or diminished before the secretion of the second shell is complete. Since nearly all of the dry shell is calcium carbonate it seemed reasonable to suppose that if such deficiency in shell-forming materials really exists it implies a deficiency of calcium which might in turn be overcome by extra feeding of soluble calcium salts. The latter possibility has been tested with a negative result in the next following paper of this series (2).

Wheeler (4) and Buckner and Martin (5) obtained some thin-shelled eggs from fowls after prolonged reduction of calcium intake, and showed that calcium is then removed from the bones for shell formation. The latter authors obtained no shell-less eggs and conclude

that "the lack of calcium is not the fundamental cause of their formation." The data obtained by Riddle and Hanke (2) seem to make this conclusion fairly certain for pigeons.

It is quite possible, of course, that not a deficiency of calcium but an unbalanced proportion of this element in relation to other elements, —phosphorus, potassium, sodium, etc., is the basis of the disorder. It is clearly possible also that the disorder is wholly unconnected with any of these or with any other substances necessary to normal nutrition. The possibility of a vitamine or other nutritional deficiency is, however, further suggested by the observation that most of the aberrant shells and embyros are produced by females which have twisted and abnormal keel bones—a condition readily suggestive of rickets. A later publication by Riddle and Rose will show that whatever the nature of this disturbance it is not corrected by the administration of any or all of the vitamines as these are prepared from yeast, skim-milk powder or spinach; nor as they exist in their normal state in orange juice, tomato juice and cod liver oil. A thorough investigation of the possible relation of nutritional deficiency to the production of thin shells and early-dying embryos is now in progress.

SUMMARY

Individual female pigeons which occasionally produce soft-shelled eggs and obviously thin-shelled eggs may produce other eggs with quite normal or even with unusually thick shells. A very high proportion of the embryos which arise in all these groups of eggs die before hatching.

The production of inadequate shells and the early death of the embryos are thus causally associated, although the relative inadequacy of a particular shell is but loosely correlated with the death of the particular embryo contained within it. An unknown and more deeply seated cause is responsible for both the occasional inadequate or irregular shells and the numerous early deaths of embryos.

Experience indicates that among pigeons the thin shells and associated early-dying embryos often occur after a long series of normal shells and viable young have been produced. This too when the same male is used throughout; the sperm cells are probably not in any way responsible for the result. When female pigeons are made to produce eggs at an abnormally rapid rate it sometimes occurs that series of embryos show the attainment of more advanced age by the earlier

embryos of the series and progressively earlier embryonic death to the end of the series.

The relative inadequacy of the shells can be measured by the relative rates of loss of water vapor through the shells. By this means of measurement the second eggs of the pair or clutch usually have the thinner shells. This is particularly true for the eggs of birds which produce some eggs with inadequate shells.

The measurement of actual amounts of dry shell material, of ash and of total inorganic bases in the ash of many eggs with presumably normal shells lends support to the view that the second eggs of the clutch are more likely to receive a slightly reduced relative amount of shell material.

Some considerations would suggest that the organism's available supply of calcium is depleted before the bird has completed the formation of two shells in rapid succession. The early death of the embyros seems, however, to indicate that something in the ovum (germ) is in disorder almost or quite simultaneously with the disordered functioning of the oviduct. Several possible nutritional deficiencies have been investigated. The real cause of the disorder in ovum and oviduct is quite unknown.

Whatever the nature of the cause of the inadequate shells and the early death of associated embryos it is clear that among pigeons, and probably also in several or all branches of the poultry industry, many individual birds which persistently fail to produce viable young may be identified and their eggs eliminated from incubation tests through the observation that they produce some eggs with soft or inadequate shells.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XI. Effects of Feeding Soluble Calcium Salts upon Reproductive Secretions and upon the Total Inorganic Constituents of the Egg Shell

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Received for publication June 6, 1921

In an earlier study (1) one of us has shown that apparently normal, though really inadequate, egg shells are responsible for the early death of an important fraction of bird embryos. Any considerable experience in working with such eggs readily leads to the conclusion that the shells are too thin and probably do not contain the normal amount of inorganic shell-substance. Since it is known that calcium carbonate forms rather more than 90 per cent of the moist shell there exists the possibility that a lack of soluble calcium in the food is associated with the production of these thin and deficient shells. The present study is chiefly an attempt to learn whether the addition of calcium lactate and calcium lactophosphate to the usual diet of ring-doves results in any increased deposition of inorganic matter in the shell.

Practically all investigations on the subject have shown an increased calcium excretion in the urine of mammals following the ingestion of inorganic lime salts. Givens (2) found the same in using calcium lactate. Earlier studies on the calcium balance under calcium lactate feeding usually demonstrated calcium retention following fairly heavy dosage. Positive results were reported for the human subject by Berg (3) who administered 3 grams daily and by Voorheve (4) who used 15 grams daily. Mendel and Givens (5) obtained a slight positive calcium balance in dogs when dosage was raised to 3.53 grams daily. Determinations of the amount of calcium in the blood following calcium lactate feeding suggest that among mammals an increase of blood calcium can be thus obtained in some species, but not in others; or possibly in the latter species only in individuals with abnormally low blood calcium. Boggs (6) obtained a decided increase in whole

blood of the dog. Halverson, Mohler and Bergeim (7) found that the calcium values of human serum are usually little affected but were increased to normal in cases of uremia and nephritis; also that the calcium excretion in the urine may be increased in certain diseases but not in others. Denis and Minot (8) failed in most cases to find an increase in the plasma of men, cats and rabbits. They further state "that in cats and rabbits where the initial concentration is low it is sometimes possible to greatly increase the amount of calcium in the plasma."

Effects of calcium lactate feeding upon reproductive functions have been reported by Emmerich and Loew (9) who stated that female mice, guinea pigs and rabbits respond to dosage by increased numbers of pregnancies and higher average numbers of embryos. Pearl (10) has made a partial report upon the result of feeding calcium lactate and also calcium lactophosphate to growing fowls. It is stated that the rate of growth of young female chicks was much increased, and that their egg laying records (later?) were greatly increased. In the latter study the dosage used seems to have been considerably larger than we have employed. In our own study there was no increase in the rate or number of ovulations.

Since the present study was concluded Buckner and Martin (11) have published results which are of very considerable interest in connection with the chief topic of the present paper. During several months Buckner and Martin withheld from laying hens all of the inorganic calcium supply which fowls usually obtain from limestone, oyster-shell grit, etc. They conclude: That such hens will continue to lay eggs until there is a general depletion of Mg, P and Ca in the bones; that egg production was much decreased; that the percentage composition of the egg shells thus obtained was not materially altered but a general thinning of the egg shells occurred; and that "since no shell-less eggs were laid it would indicate that the lack of calcium is not the fundamental cause of their formation." These investigators freed and ashed the egg shells of the fowl, much as we have done in the dove, and in addition made determinations of Ca, Mg and P in the ash. They found the calcium of the ash, calculated as CaO_2, to be about 98.0 per cent of the total ash; MgO, about 0.71 per cent; P_2O_5, about 0.70 per cent; with other undetermined elements therefore very small in amount.

From a study of calcium and phosphorus metabolism in cattle, Meigs, Blatherwick and Cary (12) obtained evidence that nervous disturbance, connected with the collection of urine, feces, etc., has a distinct effect

upon the cow's assimilation of calcium. Our own study involved this possible disturbance of shell formation but earlier work had shown that yolk and albumen secretion were certainly not thus modified.

Materials. It would at first seem an easy matter to obtain a definite answer as to whether feeding calcium salt results in the production of increased amounts of shell-substance. But in ring-dove eggs the actual variations of egg size and surface—upon which the amount of shell substance normally depends—and the possibility that the calcium salts used may themselves introduce further variations in some of the secretions—upon which again total egg size depends—unite in making the problem much less simple. We have thought it necessary to study series of eggs obtained from ten different female ring-doves, and to use three different quantities or degrees of dosage. It thus results that some at least of the several necessary measurements have had to be made upon 140 eggs. Control data, before and after calcium feeding, were obtained from 88 eggs; 2 eggs were obtained from the lightest dosage (0.113 gram, once daily, ♀ 48); 28 eggs were obtained from the same amount given twice daily and 20 eggs from heavy dosage (0.226 gram twice daily). Of the dosage mentioned 64 per cent was calcium lactate and 36 per cent was calcium lactophosphate.

The ring-doves used in these experiments had an average weight of 170 grams. The total daily amount of calcium, calculated as calcium lactate, in the lightest dosage was therefore about 0.45 gram per kilo body weight. Our lightest dosage was higher than that used in any mammalian studies with which we are familiar; but since these small birds were losing more than $\frac{1}{4}$ gram of Ca in the shells alone of the two eggs produced each 8 or 10 days, the dosages selected were considered advisable. The birds were caught twice daily and capsules containing the calcium salts were placed, usually with very slight resistance, well into the throat with artery forceps. There was no regurgitation of dosage.

Full and complete measurements were not made on all of the 140 eggs. The separation of the shell, piece by piece, from the shell membrane of the thoroughly steamed egg was a most tedious and time-consuming task; this separation was omitted for all of the later control series (after dosage). The pressure of other work also prevented the ashing of a number of prepared shells. Nevertheless, we believe an adequate amount of data is at hand. Since eleven or twelve tables would be necessary to give the details and necessary summaries of these data we have thought it advisable to present only the averages and summaries here grouped within four tables.

Presentation of data. The first point made clear by the tabulated data is that during the calcium feeding there was, in most cases, a progressive decrease in total egg size. For some individuals this continued even after dosage was discontinued. Since the absolute amount of shell-substance is closely correlated with the amount of egg surface, and this latter with egg size, it is clear that the measurements of shell weights and total ash must be interpreted in the light of this progressive decrease of egg surface. We have not attempted a direct measurement of egg surface; but, as an indirect measurement of the amount and direction of changes in surface, we have made complete records of egg weights. Although two of the ten treated females show an increase instead of a decrease in egg size, the fact remains that the average egg size was reduced; and unless the amount of inorganic material deposited per unit of surface was increased by the calcium dosage, a smaller amount of dry shell material and inorganic substance may be expected from shells obtained during the dosage period. The data from individual females show that of the eight whose egg size decreased under dosage, six show a decrease and two an increase in shell weight under dosage Of the two birds showing increase of egg weight under dosage one shows increase in shell weight while the other shows perhaps a slight decrease (tables 1 to 3). All of the averages of table 4 indicate a reduction of egg size under dosage, and also a decrease in absolute amounts of dry shell-substance. Such a result concerning the dry shell-substance found for control and treated periods indicates that the calcium feeding had little or no influence upon the absolute amount of substance utilized and laid down by the shell glands of the birds.

An important consequence of this diminution of egg size under dosage is an expected rise in the proportion of shell weight to total egg weight, since the smaller sphere or ovoid has a higher proportion of surface in relation to mass than a larger sphere. That this is of significance for even the relatively slight differences found here is made quite clear by the details of our data for 17 pairs (or clutches) of eggs used for "control" (before dosage). In 16 of these 17 pairs the shell weight of the smaller egg was the higher percentage of the total egg weight. But if comparison is made between the eggs (averaging smaller) of the "dosage period" and those of the earlier "control period" it will be found that the (smaller) treated eggs tend to have rather lower than higher values for shell material in proportion to total egg weight (next to last columns, tables 1 to 4). This necessary consideration of the results also gives therefore no indication that the calcium feeding was able to

increase the proportion of shell-substance. The proportion of dry shell material is nearly equal to, or slightly less than, the usual or expected amount.

The results for total ash are essentially comparable with those just stated for dry shell weights. The absolute amounts of ash were usually decreased (tables 1, 3, 4). Taking proper account of differences of

TABLE 1

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. A622, upper division; 907, middle division; A347, lower division of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
			Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
Control....	10/28–11/7	4	8.685	2.199	6.040	0.4456	0.2365	25.34	20.31	5.137	53.09
Smaller dosage...	11/13–11/24	4	8.243	2.155	5.682	0.4050	0.2191	26.16	18.84	4.918	54.08
Heavy dosage...	12/ 1–12/20	5	7.605	2.113	5.112	0.3801	0.2046	27.78	18.04	5.005	53.83
Control....	12/26–1 /14	5	7.620	2.125				27.84			
Control....	10/28–11/ 9	4	9.064	1.932	6.692	0.4641	0.2463	21.31	24.06	5.122	53.06
Smaller dosage...	11/18–11/20	4	9.118	2.041	6.608	0.4698	0.2536	22.34	23.11	5.153	53.66
Heavy dosage...	12/ 8–12/20	4	9.110	1.959	6.671	0.4797	0.2583	21.52	24.54	5.266	53.84
Control....	12/27– 1/8	4	9.206	1.972				21.40			
Control....	11/ 2–11/13	4	8.406	1.961	6.064	0.3805	0.1975	23.26	19.63	4.536	51.88
Smaller dosage...	11/21–12/3	4	8.019	1.884	5.775	0.3604	0.1913	23.46	19.19	4.493	53.05
Heavy dosage...	12/12–12/14	2	8.084	2.028	5.698	0.3578	0.1897	25.05	17.70	4.427	53.01
Control....	12/22– 1/12	6	8.030	1.895				23.58			

egg size it is only in the eggs of one of the four birds for which sufficient data were obtained (♀ 907, table 1) that the results can be interpreted as indicating an increase in total amount of inorganic matter under the calcium dosage.

On the other hand, several averages given indicate that the *percentage* of ash in the dry shell substance is higher in shells derived from the

dosage periods. Seven group comparisons are possible—three of groups from heavy dosage compared with control (table 1, last column) and four of groups from smaller dosage compared with control. Of these seven groups only one (from smaller dosage, ♀ K459, table 3) gives a lower percentage of ash under dosage. The summary given in the

TABLE 2

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. A120, upper division; E317, middle division; A843 lower division of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS						PERCENTAGE RELATIONS OF PARTS		
			Egg	Yolk	Albumen	Shell		Ash	Yolk to egg	Shell to yolk	Shell to egg
						Moist	Dry				
Control..	11/ 2–11/12	4	8.677	1.961	6.268	0.4528	0.4478		22.53	23.19	5.174
Smaller dosage.	12 /4–12/6	2	8.444	1.936	6.060	0.4521	0.4477		22.94	23.15	5.301
Heavy dosage.	12/18–12/20	2	8.316	1.829	6.036	0.4563	0.4518		21.98	24.78	5.436
Control..	12/30– 1/19	6	8.613	1.896					21.96		
Control..	11/ 2–11/26	4	8.978	1.682	6.878	0.4238	0.4187	0.2282*	18.72	25.00	4.672
Smaller dosage.	11/26–12/9	1	8.718	1.773	6.534	0.4157	0.4108		20.33	23.17	4.712
Heavy dosage.	12/10–12/20	1	8.886	1.669	6.841	0.3807	0.3756		18.78	22.27	4.286
Control..	12/28– 1/31	5	8.698	1.603					18.44		
Control..	11/ 1–11/15	4	8.873	2.239	6.224	0.4152	0.4102	0.2153[1]	25.23	18.34	4.627
Smaller dosage.	11/30–12/13	3	8.576	2.178	5.981	0.4219	0.4165		25.39	19.22	4.855
Control..	12/23– 1/5	4	8.711	2.113					24.26		

* For a single egg whose weight was 8.640 grams; shell (dry), 0.4271 gram; and whose ash was 53.43 per cent of the shell.

[1] For a single egg whose weight was 9.022 grams; shell (dry), 0.3981 gram; and whose ash was 54.08 per cent of the shell.

last column of table 4 indicates that the dry weight of shells produced under the calcium dosage yields 53.7 per cent of ash as compared with 52.7 per cent from normal shells. However, inspection of the last column of table 3 will show that three birds—not included in any averages because none of the shells from the dosage period was ashed—have

quite high percentages for the control period. Also, it seems that low percentages in control usually gave higher percentages under dosage, and vice versa. The seeming difference in ash percentage just noted is, therefore, probably insignificant.

TABLE 3

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. K459, upper division; 152 and A798 next divisions; 48, lower divisions of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
			Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
Control..	11/ 6–11/ 8	2	7.548	1.700	5.496	0.3530	0.1891	22.51	20.87	4.695	53.57
Smaller dosage.	11/17–12/20	6	7.721	1.817	5.552	0.3524	0.1856	23.47	19.60	4.573	52.83
Control..	12/29– 1/21	6	7.982	1.870				23.35			
Control..	11/12–11/26	4	3.325	1.986	5.926	0.4132	0.2196	23.81	20.94	4.966	53.68
Smaller dosage.	12/11–12/13	2	8.145	2.000	5.742	0.4038		24.55	20.23	4.959	
Control..	12/25– 1/7	4	8.245	1.859				22.53			
Control..	10/30–11/11	4	10.243	2.434	7.333	0.4770	0.2492*	23.69	19.90	4.681	53.85*
Smaller dosage.	11/27–11/29	2	9.809	2.306	7.030	0.4733		23.46	20.58	4.824	
Heavy dosage.	12/28– 1/27	6	10.008	2.300				22.96			
Control..	11/12–11/28	3	8.367	2.050	5.858	0.4586	0.2562[1]	24.50	22.45	5.481	54.38[1]
Lightest dosage.	12/ 6–12/8	2	7.822	1.969	5.451	0.4028		25.15	20.51	5.156	
Control..	12/29– 2/2	5	7.711	1.821				23.04			

* Average for two eggs whose weight was 10.410 grams; shell (dry), 0.4627 gram.
[1] For a single egg whose weight was 8.561 grams; shell (dry), 0.4711 gram.

A decrease in egg size during the calcium feeding has already been noted. It remains to locate the part or parts of the egg which undergo this reduction. The size of the enclosed yolk is ordinarily and normally the chief, though indirect, determining factor in ultimate egg weight. The data show that there was perhaps a slight decrease in yolk size during the dosage period. But an examination of the average weights

of "yolk" and "albumen" in table 4 clearly shows that, even in cases where yolk weight was fully maintained in dosage periods, the albumen

TABLE 4

Summaries (weighted averages throughout) of effects of smaller and heavier dosage of calcium lactate and calcium lactophosphate on the various egg-structures of ring-doves (A622, 907, A347, E317 and A120 in upper division of table; all included in lower division of table)

TREATMENT	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
		Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
For five birds (smaller and heavy dosage)										
Control	20	8.762	1.947	6.3884	0.4323		22.23	22.43	4.928	
Smaller dosage	15	8.474	2.008	6.0609	0.4164		23.60	20.92	4.904	
Heavy dosage	14	8.297	1.983	5.8960	0.4224		24.13	21.11	5.007	
Control	26	8.394	1.941				22.66			
Control[1]	12	8.718	2.031	6.265	0.4301	0.2268	23.30	21.18	4.933	52.73
Smaller dosage[1]	12	8.460	2.027	6.022	0.4117	0.2211	23.96	20.31	4.866	53.70
Heavy dosage[1]	11	8.238	2.042	5.785	0.4123	0.2214	24.79	20.29	5.005	53.70
For ten birds (smaller dosage only)										
Control	37	8.785	2.025	6.331	0.4300		23.05	21.23	4.895	
Smaller dosage	30	8.358	1.998	5.889	0.4060		23.91	20.32	4.858	
Control	51	8.483	1.927				22.72			
Control[2]	14	8.540	1.983	6.155	0.4191	0.2214	23.22	21.13	4.907	52.45
Smaller dosage[2]	18	8.214	1.980	5.865	0.3918	0.2094	24.11	19.79	4.770	53.45
Control[2]	21	8.143	1.960				24.07			

[1] This part of summary—including *ash* determinations for the three series—can be given for only three (A622, 907 and A347) of the five females.

[2] This part of summary—including *ash* determinations—can be given for only four (A347, K459, 907 and A622) of the ten females.

fell plainly below the control and that practically the whole of the loss of egg weight is due to the presence of distinctly less albumen. The slight reduction in average yolk size is almost certainly attributable to

another circumstance.[1] In our opinion the marked reduction of albumen secreted in the dosage periods is, in one way or another, attributable to the addition of the calcium salts to the diet.

The ten birds that have been described were not birds which had produced, nor were then producing, obviously thin-shelled eggs. However, the details of our data show that the control eggs of any individual bird were unequally provided with total shell-substance and with inorganic matter. In those cases therefore it would seem that a larger supply of the necessary inorganic matter used in shell formation might assist in raising the slightly lighter shells to the level of the heavier ones. In addition, two females which were producing soft, thin or obviously defective shells were dosed (0.226 gram daily) for a period in order to learn whether normal shells might be thus produced. Neither of these birds produced any eggs during 3 weeks of dosage; their dosage was therefore discontinued. In two other tests of similar females, with dosage extended to 1–3 months, the production of eggs with soft or thin shells and early-dying embryos was continued.

DISCUSSION

As results of this study two points are fairly clear. First, that the amount of inorganic substance laid down in the egg shells was practically unchanged by the extra calcium intake. The dry weight of shells produced during dosage seems slightly under normal weight, while an equal or insignificantly higher percentage of ash is present in these slightly lighter shells. Second, the unexpected circumstance that the extra calcium feeding resulted in a reduced secretion of albumen.

Disturbances incident to the catching and dosage of our birds, and above all a conceivable degree of nausea from the calcium salt in the crop, may have tended toward a *reduced* calcium assimilation. This inference could be drawn from the work of Meigs, Blatherwick and Cary cited above. Also, if the salt produced any lack of appetite—which would have escaped observation—the normal source of calcium may have been thus reduced. These assumptions could be considered in connection with the failure of our calcium feeding to increase the amount of shell-substance if they could better meet the difficulties in

[1] Females A798 and 48 failed to produce eggs continuously under dosage and therefore obtained a period of "reproductive rest." Such a period of rest has been previously found to be normally followed by yolks of smaller size. Female F317 obtained partial periods of rest and produced only one *pair* of eggs, and five *unpaired* eggs after the early "control" data.

explaining the observed changes in the albumen. Disturbances incident to the handling of the birds certainly can not account for the decreased production of albumen, since wholly similar birds were caught and blank-dosed in connection with an earlier study and it was learned that no change is thus produced. That nausea or under-feeding could produce a decreased secretion of albumen is perhaps not improbable. But the average body weights of the birds remained practically constant under dosage, and this fact is difficult to reconcile with any under-feeding during dosage. Again, the rate of egg-production was unchanged.

Our own observations on the condition of our experimental animals, and the results of similar earlier investigations, permit us to consider it highly probable that these doves absorbed and excreted more calcium while under dosage; our data demonstrate nevertheless that little or no change was produced in the amount of material deposited in the shell. That the maximum of shell production was not attained, neither in the control nor in the calcium dosage periods, is plainly evident from the details obtained for individual eggs and from other observations and experience as well. Our results therefore support Buchner and Martin's conclusion that the lack of calcium is not the fundamental cause of the production of thin-shelled eggs. Their conclusion was based upon data derived from under-feeding of inorganic calcium to fowls; our conclusion is based upon extra feeding of soluble calcium to ring-doves.

Wheeler (13) found, somewhat earlier than Buckner and Martin, that a long continuance of a diet low in calcium results in the production by fowls of some eggs with thin shells; and that calcium is earlier freely removed from the bones for shell formation. Wheeler also made the further interesting observation that strontium can very largely replace calcium in both the shell and bones of fowls and ducks, though magnesium is incapable of doing so.

On the puzzling result concerning the reduced albumen secretion observed by us we can offer only the following suggestion. If a sufficient excess of calcium were present in the circulation, the well-known action of calcium might have effected a depression of the nerve, muscle or gland cells of the albumen-secreting gland. Any muscular effect which would permit or induce a more rapid transit of the egg through the albumen-secreting part of the oviduct (the total time of passage through the entire oviduct was certainly not modified), or a diminished activity of the albumen secreting cells, or a general reduction of the body metabo-

lism, could account for the decrease actually observed. It seems less probable that the time spent in the albumen-secreting gland was shortened than that the normal rate of activity of the gland was reduced.

SUMMARY

Calcium lactate and calcium lactophosphate were added in various amounts to the normal diet of freely laying ring-doves. The possible quantitative changes in the shell and in other gross egg constituents were measured.

The amount of dry shell-substance was not increased, but perhaps slightly diminished, under the extra calcium feeding. The percentage of inorganic matter in the dry shell-substance was probably unchanged.

The amount of albumen secreted under extra calcium feeding was measurably decreased.

No measurable change in the rate of reproduction (ovulation) occurred in the treated birds.

The production of inadequate shells, or of thin-shelled eggs, which is associated with the early death of many bird embryos is probably not caused by an inadequate calcium supply in the food. The feeding of organic calcium salts to female ring-doves failed appreciably to strengthen their shells.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XII. The Relation of Nerve Stimuli to Oviducal Secretions as Indicated by Effects of Atropine and Other Alkaloids

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Received for publication June 6, 1921

The oviduct of birds contains three glands whose markedly intermittent activity gives rise to three different and highly specialized products—egg-albumen, shell membrane and egg shell. The relation of nerve stimuli to this intermittent glandular activity is apparently quite unknown. It is well known, however, that the presence or placement of solid or semi-solid objects within the upper oviduct usually, but not invariably, results in the secretion of albumen, membrane and shell. Such an introduced object may be an egg yolk or any one of many substitutes such as feathers or dirt caught up by the everted oviduct, an amber bead (1), a plug of wood or rubber (2), a mass of feces or of agar (3). Observations of this sort give support to the view that these glandular secretions occur in response to contact, mechanical or pressure stimuli necessarily connected with the presence of the foreign object; but whether local reflexes are also directly involved is a matter wholly in doubt.

Of much importance in this situation is the fact that introduced foreign bodies can induce these secretions only in an oviduct which is in an active or functional state; and that this functional state is attained as a result of, or in close association with, processes occurring in the ovary. An internal secretion is therefore apparently involved in this preliminary preparation or stimulation of the gland. Whether this same hypothetical internal secretion which prepares the gland for activity is also an active or effective agent in inducing the actual secretion, once a contact stimulus is supplied, is a question really unanswered by such data as are now available for the bird.

The possibility of nervous control of these secretions certainly exists, though the necessary specific facts are at present lacking. Unfortunately, we have been unable to find any definite anatomic knowledge of the innervation of the glands of the avian oviduct; and physiological or pharmacological studies on these nerves of the bird seem never to have been attempted. In the absence of definite information one may nevertheless begin an investigation upon the following assumptions: First, that the innervation of the oviduct is similar to that of the homologous organ—the uterus—of mammals. Even among amphibians Langley and Orbelli (4) found "that in its general features the plan of the sympathetic innervation of the viscera of the frog is the same as that of the mammal." Second, that the highly glandular part as well as the muscular part may have received parasympathetic and sympathetic innervation in birds. Third, that the action of atropine and other alkaloids is likely to be rather similar in the two organs. If these assumptions approximately represent the facts we may reasonably hope to decrease or to increase the oviducal secretions of the dove by means of proper doses of those alkaloids which have been found markedly to affect secretions under the control of autonomic nerves in mammals; one may, for example, expect to diminish the secretions by atropine.

It is understood, however, that a decreased secretion of albumen or of shell would not be a necessary result of the depressant action of atropine since it is known that parasympathetic depression or stimulation does not respectively decrease or increase the output of all glands having this innervation. And until now apparently we have little or no data by which thus to classify the oviducal glands of the bird. Again, there is the possibility that any observed effect on the amount of secretion under the drug may be the result of the action of the latter on the gland cells themselves. In view of these and still other uncertainties we shall here confine ourselves largely to an objective description of our results. When further facts shall have been accumulated a fuller interpretation may be possible. Also, other investigators may be in possession of information which has not been available to us. Since, however, we have made use of drugs—atropine, cocaine, nicotine, pilocarpine—which are reputed to have such characteristic action on the autonomic nerves of the mammal it seems to us highly probable that our results throw some light upon the extent to which the secretion of albumen and shell are dependent upon nerve stimuli. In any case our data supply a measure of the effect or lack of effect of particular dosages of these drugs upon these secretions of the oviduct.

Our special reason for undertaking this study was our need of the results of this inquiry in a series of studies whose object is to learn why the eggs of some birds are habitually or sporadically provided with thin or inadequate shells. That general problem has been discussed earlier by one of us (5). In the immediately preceding paper of this series (6) it was found that an increased intake of soluble calcium salts has very little effect upon the amount of calcium laid down in the shell. The question next arose: Is the secretion of the shell under the control of nerves? Are the egg shells of birds sometimes inadequate because of nervous deficiency or derangement? The present study has been carried out with particular reference to a decision of this point. If, however, the secretion of shell and albumen are found to be not really under nervous control the question of the possible mechanisms which play a part in regulating the intermittent activity of those glands is placed one step nearer solution,—since, in that case, direct contact stimuli and the influence of an internal secretion seem the only other alternatives.

Materials and methods. Eight female ring-doves (blond and white ring-dove hybrids) which were producing pairs of eggs (clutches) at intervals of 6 to 8 days were selected for study. These birds had been previously fed calcium lactate during a period of a month or more, as reported elsewhere (6). A period of 40 to 60 days elapsed after the lactate feeding before the treatment here recorded was begun. These birds had an average body weight of 169 grams at the beginning and 165 grams at the close of atropine administration. Body weight after the short cocaine, nicotine and pilocarpine dosage was not determined.

Atropine sulfate (Parke, Davis Co.) was administered in four dosages. The lightest dosage was of 0.002 grain, once daily, tested on a single bird (♀48, table 3). Other dosages were: 0.002 grain (7 birds), 0.005 grain (7 birds), 0.01 grain (2 birds); all given twice daily at about 9:00 a. m. and 7:00 p. m. The heavier dosages were given to birds which had earlier been given lighter dosage.[1] Willburg (7) determined the lethal dosage of subcutaneously injected atropine sulfate in a number of animals; for the dove he found this to be 0.2 to 0.25 gram per kilogram body weight. The dove was found 113 times more resistant to the drug than is man. In our administration of the drug the relatively tame birds were caught and tablets of appropriate size dropped far back into the throat. Birds were frequently watched for regurgitation; none was ever noted. Four birds (table 4) were given 0.2 and 0.4 grain atropine thrice daily.

[1] For the extremely heavy dosage of atropine and pilocarpine (table 4) another group of eight females was used. These birds had not previously been treated with any drug.

The only supply of nicotine (Merck) available to us had been purchased nearly six years before using. It had been kept unopened in the dark and its strength was tested as follows: A dove of 175 grams body weight, injected subcutaneously with 1.67 mgm. died in 1 to 1½ minutes. A similar dove given 0.67 mgm. vomited, showed slight diarrhea, and distinctly heavier respiration. Within ½ hour the dosage to this bird was increased by 0.57 mgm. The above symptoms (except diarrhea) increased, eyelid movements became abnormal and standing position unsteady. A third dove survived a dosage of 1.0 mgm. but showed many signs of collapse. All nicotine solutions were made up in water each 3 days and kept in the dark. The strength of the solutions was made such as to require subcutaneous injections of only ½ cc.

Cocaine (Boehringer) was converted by us into the hydrochloride and subcutaneously injected in aqueous solution.

Pilocarpine hydrochloride (U. S. P.) was given per os as was the atropine.

None of the above drugs was given continuously, but solely with reference to the time of egg-laying. These doves lay two eggs in a clutch, the two being laid 40 hours apart. The first of the pair is laid at very nearly 5:00 p.m.; the second at nearly 9:00 a.m. of the second morning thereafter. When the eggs are removed from the nest immediately after laying, the first egg of a next following pair may usually be expected 6 or 7 days thereafter. The egg requires about 45 hours for passage down the oviduct. An egg laid 6 days after the last of the previous pair would therefore leave the ovary and begin to receive the oviducal secretions about 4 days after the laying of the last preceding egg.

Since our purpose was not to study effects of the dosage upon the work of the ovary (yolk size),[2] and in order to avoid the probable cessation of egg production incident to continuous dosage, atropine was given only from the morning of the 3rd day after the laying of the last of the preceding pair of eggs. The albumen is secreted during the first 15 of the 45 hours spent by the egg in the oviduct; the formation of the shell occurs during the last 30 hours. The administration of nicotine and of cocaine was so timed as to affect the secretion of the shell only, of the first egg of the prospective pair; this, however, necessarily subjected the albumen secretion of the second egg of the pair to the action of the drug.

The eggs and shells were prepared in the following manner: Eggs were obtained 10 minutes to 3 hours after laying; they were weighed, steamed for 8 minutes, cooled in tap water for 4 minutes and the shell removed piece by piece with small curved-tip forceps. The weights of the solid coagulated yolk and of the moist (nearly air dry) shell were next obtained. The sum of these two weights subtracted from the total egg weight gave the weight of the albumen (including shell membrane). The albumen cannot be weighed directly on account of evaporation during removal of the shell.

Since nearly all of the inorganic material of the shell is in the form of calcium carbonate[3]—with magnesium carbonate and earthy phosphates present in very

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[2] Unavoidably, however, the *yolk* of the first of the pair was subjected during its last 1 to 2 days in the ovary to the action of the drug; while the *yolk* of the second of the pair was thus affected during the last 3 to 4 days of its growth in the ovary.

[3] Buckner and Martin (8) found in shells of fowls' eggs that the calcium calculated as CaO, is equal to about 98.0 per cent of the ash.

small amounts and representing practically all of the remainder—it was found practicable and desirable, because of the numerous samples, to make volumetric determinations of the total of these alkaline earths. The egg shell was dried in a weighing bottle for 12 hours at 105°C. It was found that the organic matrix of the shell prevented ready solubility in weak acid. Since the nature of the procedure made it inadvisable to use a strong acid the dry shell was transferred to a porcelain crucible and ignited until the small amount of organic material had been removed and then over a heavy flame until all carbonate was converted into oxide. The crucible and ash were then placed in a beaker and the ash dissolved in 100 cc. N/10 HCl. By using 100 cc. there was an excess of 10 to 40 cc. which was determined by titration with N/10 NaOH. Methyl orange was used as indicator. The values for total bases thus obtained are considered as wholly calcium and calculated in the tables as per cent $CaCO_3$ in dry shell.

Presentation of data. For obvious reasons it seemed best to study the effects of the drugs chiefly—though not exclusively—on birds whose secretions were normal and to place chief reliance upon a decreased secretion under atropine.

The details of data for individual egg-shells and albumens as obtained from one bird are fully given in table 1. In order to economize space other records (tables 2 and 3) are given in summary only. Reasons for inclusion of yolk size and other data in these tables are given in a preceding paper (5), and the discussion given there of the relations which normally obtain between yolk size on the one hand and the volume of the oviducal secretions on the other are necessary to a proper estimate of the present data.

Atropine. The summary given at the bottom of table 3 makes it clear that under atropine dosage the amount of albumen secreted is slightly though certainly reduced. The reduction is not more than 2 or 3 per cent. Analyses of this albumen show (table 5) that it was nearly or quite normal in respect to the relative proportions of water, alcohol-soluble and alcohol-insoluble constituents.

The amount of shell material secreted under atropine was either unaffected or but slightly affected. In this evaluation the smaller amount of egg surface presented by the eggs obtained under atropine is of some importance as shown by reference to the figures for "percentage relations of shell to egg" (tables 1 to 3). The percentage of alkaline earths present in the shell-ash was possibly slightly reduced under atropine. The difference involved is of questionable value.

The two birds (table 1; and ♀907, table 2) which each received three different degrees of atropine dosage do not plainly show a greater effect of the dosage with 0.02 grain daily than with 0.004 grain daily. Yolk size was unaffected by the short part of its growth period during

TABLE 1

Effects of atropine and of nicotine on the oviducal secretion of ring-dove A120

TREATMENT	DATE OF EGG	AVERAGE WEIGHT IN GRAMS					PER CENT OF BASES IN SHELL ASH ($CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
		Egg	Yolk	Albumen	Shell			Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
					Moist	Dry					
Control	1/26	8.581	2.018	6.119	0.4440	0.4361	95.51	23.52	32.98	5.082	21.61
	1/28	8.761	2.230	6.133	0.3982	0.3918	95.13	25.45	36.36	4.472	17.57
	2/4	8.771	1.954	6.382	0.4350	0.4285	95.81	22.28	30.62	4.885	21.93
	2/13	9.409	2.077	6.841	0.4913	0.4813	95.40	22.08	30.36	5.115	23.17
	Average.	*8.881*	*2.070*	*6.369*	*0.4422*	*0.4344*	*95.46*	*23.33*	*32.58*	*4.889*	*21.07*
Atropine.	2/20*	8.250	1.892	5.990	0.3679	0.3638	94.60	22.93	31.59	4.410	19.23
	3/1	8.194	1.885	5.854	0.4552	0.4464	95.44	23.00	32.20	5.448	23.68
	3/3	8.826	2.150	6.231	0.4453	0.4320	95.53	24.36	34.50	4.895	20.09
	Average.	*8.423*	*1.976*	*6.025*	*0.4228*	*0.4141*	*95.19*	*23.43*	*32.76*	*4.918*	*21.00*
Atropine	3/10[1]	8.925	2.019	6.438	0.4678	0.4556	94.63	22.62	31.36	5.105	22.57
	3/12	8.844	2.090	6.315	0.4385	0.4241	95.63	23.63	33.10	4.795	20.29
	3/19	8.793	1.951	6.371	0.4710	0.4535	95.30	22.19	29.24	5.158	23.25
	3/21	9.071	2.141	6.493	0.4372	0.4277	94.32	23.60	32.97	4.715	19.98
	Average.	*8.908*	*2.050*	*6.404*	*0.4536*	*0.4402*	*94.97*	*23.01*	*31.67*	*4.943*	*21.53*
Atropine	3/28[2]	8.659	1.901	6.308	0.4495	0.4412	95.27	21.95	30.14	5.095	23.21
	3/30	8.875	2.089	6.366	0.4201	0.4089	95.40	23.54	32.81	4.607	19.57
	4/5	7.991	1.609	5.952	0.4297	0.4181	96.37	20.14	27.03	5.232	25.99
	4/7	8.820	1.942	6.449	0.4287	0.4134	96.00	22.02	30.11	4.687	21.29
	Average.	*8.586*	*1.885*	*6.269*	*0.4320*	*0.4204*	*95.76*	*21.91*	*30.02*	*4.905*	*22.52*
Control	4/13	8.558	1.741	6.341	0.4761	0.4644	95.81	20.34	27.39	5.427	26.67
	4/15	9.202	2.092	6.680	0.4304	0.4170	95.59	22.73	31.32	4.532	19.93
	4/21	8.430	1.783	6.185	0.4617	0.4513	95.30	21.15	29.50	5.354	25.31
	4/23	8.874	2.035	6.401	0.4379	0.4206	96.20	22.93	31.79	4.740	20.67
	Average.	*8.766*	*1.913*	*6.402*	*0.4515*	*0.4383*	*95.72*	*21.79*	*30.00*	*5.013*	*23.14*
Nicotine	5/9[3]	7.987	1.659	5.899	0.4247	0.4120	93.68	20.77	28.12	5.158	24.83
	5/17[4]	8.516	1.729	6.275	0.5115	0.4954	95.72	20.30	27.55	5.817	28.65
	5/19	8.583	1.805	6.312	0.4658	0.4414	96.21	21.03	28.60	5.143	24.45
	Average.	*8.362*	*1.731*	*6.162*	*0.4673*	*0.4496*	*95.20*	*20.70*	*28.09*	*5.373*	*25.98*
Control	5/26	8.726	1.936					22.19			
	5/28	9.201	2.129					23.14			
	6/3	8.723	1.898					21.76			
	6/5	8.766	2.035					23.21			
	Average.	*8.854*	*2.000*					*22.58*			

* Atropine dosage (0.002 grain twice daily) began on 2/16.

[1] Heavy dosage (0.005 grain twice daily) from 3/6 to 3/21.

[2] Extra heavy dosage (0.01 grain twice daily) from 3/24 to 4/6

[3] Injections of nicotine (0.19 mgm. once daily, at 12:45 p. m.—given from 5/8 to 5/10.

[4] Injections of nicotine (0.19 mgm. each); 1 on 5/16, 2 on 5/17, 2 on 5/18.

TABLE 2

Effects of atropine, cocaine and nicotine on the oviducal secretions of ring-doves 907, A798, A843, 152.

(Birds in this order from top of table)

TREATMENT	DATA ON EGGS		AVERAGE WEIGHT IN GRAMS				PER CENT OF BASES IN SHELL ASH (AS $CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
	Number	Date	Egg	Yolk	Albumen	Shell (dry)		Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
Control....	6	1/24–2/12	9.755	2.051	7.204	0.4567	95.51	21.01	29.05	4.681	22.34
Atropine*..	4	2/19–3/2	9.356	2.067	6.835	0.4465	95.41	22.10	30.26	4.774	21.61
Atropine[1]..	4	3/10–3/31	8.765	2.022	6.284	0.4523	95.29	23.06	30.20	5.157	22.43
Atropine[2]..	4	3/28–4/8	8.698	2.015	6.244	0.4322	95.39	23.16	32.28	4.972	21.47
Control....	4	4/14–4/25	8.919	2.036	6.432	0.4401	95.69	22.77	31.75	4.935	21.73
Nicotine[3]...	1	5/2	9.009	1.958	6.633[3]	0.4096	95.61	21.73	29.52[3]	4.547	20.91
Control....	3	5/16–5/24	8.031	1.939				24.23			
Control....	4	1/25–2/9	9.930	2.290	7.185	0.4481	95.74	23.04	31.85	4.521	19.63
Atropine*..	2	2/16–2/18	10.136	2.407	7.322	0.4031	95.38	23.69	32.78	4.016	17.05
Atropine[1]..	4	3/24–4/5	9.330	2.040	6.821	0.4612	95.62	21.86	29.91	4.949	22.58
Control....	6	4/13–5/3	9.826	2.196	7.201	0.4185	95.07	22.32	30.47	4.293	19.28
Nicotine[4]..	4	5/12–5/23	9.491	2.095	6.909	0.3980	95.36	22.05	30.00	4.216	19.19
Control....	4	5/30–6/10	9.881	2.144				21.71			
Control....	4	1/27–2/9	8.694	2.082	6.193	0.4081	95.98	23.91	33.62	4.699	19.70
Atropine*..	4	2/19–3/3	8.728	2.244	6.084	0.3916	95.62	25.71	36.88	4.490	17.48
Atropine[1]..	4	3/11–3/24	8.740	2.144	6.218	0.3698	94.95	24.51	34.46	4.238	17.35
Control....	4	3/31–4/11	9.012	2.162	6.464	0.3734	95.54	23.99	33.45	4.144	17.29
Cocaine[5]...	5	4/18–5/7	8.681	2.089	6.239	0.3477	95.25	24.01	33.59	4.001	16.64
Nicotine[6]..	4	5/15–5/26	8.559	2.126	6.028	0.3832	95.91	24.86	35.33	4.483	18.05
Control....	4	6/ 2–6/13	8.919	2.168				24.28			
Control....	4	1/28–2/10	8.111	1.869	5.859	0.3761	95.75	23.00	31.90	4.635	20.17
Atropine*..	4	2/17–2/29	7.848	1.822	5.662	0.3573	94.90	23.20	32.18	4.550	19.67
Atropine[1]..	4	3/ 7–3/18	7.841	1.869	5.606	0.3492	95.28	23.86	33.34	4.592	19.31
Control[7]...	4	3/25–4/4	7.830	1.768	5.707[7]	0.3480	95.39	22.79	30.98	4.494	19.77
Cocaine[8]...	6	4/18–5/9	7.600	1.765	5.492	0.3341	95.74	23.29	32.33	4.397	18.98
Control	2	5/16–5/18	7.993	1.735	5.889	0.3541	96.25	21.70	29.45	4.434	20.45

*Atropine dosage of 0.002 grain twice daily.

[1] The dosage was 0.005 grain twice daily.

[2] Atropine dosage of 0.01 grain twice daily began 3/24, last dose given 4/7 p. m.

[3] Dosed with nicotine (0.2 mgm.) 5/1 p.m. and 5/2 a. m.; albumen secretion already complete.

[4] For the first pair of eggs the dosage was 0.19 mgm. of nicotine injected once daily from 3 days before first of pair was laid; for the second pair of eggs the dosage was 0.38 mgm. once daily beginning at 12:45 p. m. on the date the first of the pair was laid.

[5] Injections of 0.6 mgm. cocaine thrice daily for first pair, beginning 1 day before laying of first egg; for next (single) egg, 0.5 mgm. thrice daily; for last pair of eggs the same dosage beginning 2 days before laying first of pair.

[6] Injections of 0.19 mgm. nicotine once daily for first pair; of 0.38 mgm. once daily for second pair; first injection 2 days before first egg in both cases.

[7] Atropine dosage discontinued on 3/23—only 2 days before laying first egg of this control series.

[8] Three injections daily of 0.6 mgm. cocaine hydrochloride for first pair of eggs; 0.5 mgm. for last four eggs. For two pairs injections began 1 day before first egg; for third pair, 3 days earlier.

TABLE 3

Summary of effects of atropine on the oviducal secretion of ring-doves A622, 48, E317. (In this order from top of table)

TREATMENT	DATA ON EGGS		AVERAGE WEIGHT IN GRAMS				PER CENT OF BASES IN SHELL ASH (AS $CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
	Number	Date	Egg	Yolk	Albumen	Shell (dry)		Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
Control...	2	1/28–2/5	7.061	2.086	4.616	0.3533	95.23	29.54	45.19	5.001	16.92
Atropine*.	4	2/13–2/28	6.801	2.161	4.282	0.3528	95.47	31.87	50.47	5.184	16.33
Atropine[1].	5	3/7–3/23	6.677	2.088	4.232	0.3492	94.81	31.24	49.34	5.217	16.71
Control...	5	3/29–4/14	6.721	2.023	4.355	0.3331	95.29	30.10	46.45	4.963	16.49
Control...	3	1/31–2/8	7.960	1.877	5.643	0.4328	95.94	23.56	33.26	5.439	23.17
Atropine[2].	5	2/17–3/19	7.855	(1.874)	5.576	0.3936	95.69	23.84	33.60	5.042	21.21
Control...	5	3/25–4/15	8.201	2.151	5.677	0.3623	(94.80)	26.21	37.88	4.415	16.90
Control...	4	1/29–2/10	8.477	1.620	6.415	0.4072	95.74	19.09	25.25	4.805	25.20
Atropine*.	2	2/18–2/28	8.313	1.715	6.222	0.3700	95.37	20.63	27.56	4.448	21.59
Atropine[1].	3	3/7–3/17	7.861	1.488	6.009	0.3557	94.74	18.93	24.76	4.513	23.92
Control..		3/26[3]	7.823	1.695	5.729	0.3902	Lost	21.67	29.59	4.988	23.02
		4/3[3]	broken			0.4245	93.69				
		4/9[3]	7.508	1.431	5.726	0.3449	91.48	19.06	24.99	4.594	24.10
	5–6	4/14	6.683	1.438	4.955	0.2845	94.49	21.52	29.02	4.257	19.71
		4/24[3]	6.992	1.462	5.154	0.3665	94.57	20.91	28.36	5.242	25.00
		4/30[3]	7.370	1.411	5.547	0.4008	94.89	19.15	25.44	5.438	28.41
		Average.	*7.275*	*1.487*	*5.422*	*0.3686*	*93.82*	*20.46*	*27.43*	*4.904*	*24.05*
Summary[4]											
Control..	22		9.144	2.062	6.601	0.4276	95.67	22.69	31.55	4.685	20.74
Atropine..	17		8.781	2.075	6.297	0.4018	95.29	23.63	33.01	4.591	19.54
Atropine..	20		8.717	2.025	6.267	0.4125	95.22	23.26	31.92	4.776	20.64
Control..	22		8.957	2.032	6.510	0.4050	95.44	22.69	31.25	4.550	20.06

* Atropine dosage of 0.002 grain twice daily.

[1] Atropine dosage increased to 0.005 grain (twice daily) from 3/3 a. m. to 3/23.

[2] Atropine dosage (0.002 grain *once* daily) began on 2/13; not dosed after 3/23.

[3] This egg abnormally delayed in lower oviduct.

[4] The three birds whose records are given immediately above on this table either ceased producing eggs (48), or laid only abnormal eggs (A622,E317), very soon after the above records. This summary therefore excludes these three records. Also the highest dosage of atropine cannot be represented here since this dosage was given to only two of the five birds included in the summary.

TABLE 4

The effects of pilocarpine (and very heavy atropine) dosage on the thickness of shells as measured by hourly rate of loss

NUMBER OF BIRD	DOSAGE		DATE OF EGGS	TIME ACTUALLY LAID (1STS = P.M.) (2NDS = A.M.)	TREATED EGGS		MEANS FOR CONTROL EGGS	
	Amount	Time before first egg			Weight	Rate of loss	Weight	Rate of loss
	grains	*hours*			*grams*	*mgm.*	*grams*	
P904	(0.04)	20	3/18	Before 5:45	8.11	3.6	8.69	3.3
			3/20	Before 7:45	9.17	5.9	9.69	7.0
P891	(0.04)	20	3/19	4:45–5:10	7.80	3.5	8.05	(12.6)[3]
			3/21	8:30–9:00	8.41	([3])	7.84	
P900	(0.02)*	0	3/18	Before 5:30	8.60	3.3	8.56	3.4
			3/20	10:00–10:45	9.89	4.0	9.86	5.5
A250	(0.02)*	20	3/19	4:45–5:05	8.08	3.3	8.24	3.5
			3/21	8:30–9:00	9.62	5.0	9.38	4.5
P839[1]	(0.04)*	0	3/19	At 4:40	6.65	3.3	6.85	3.6
			3/21	At 9:20	7.58	7.6	7.29	10.4
P692	(0.04)*	0	3/19	At 4:49	7.23	3.5	7.22	3.4
			3/21	9:30–10:00	8.05	5.1	8.19	4.4
P830	(0.06)	0	3/20	4:10–4:40	9.66	3.7	9.25	3.6
			3/22	Before 7:45	9.89	3.1	9.88	4.2
P853[1]	(0.06)	0	3/20	At 4:39	8.99	([3])		
			3/22	12:00–2:35	9.20	([3])		
P708	(0.04)[2]	0	3/25	Before 9:40	7.58	4.8	8.20	6.5
			3/27	Before 11:30	8.90	7.6	8.44	6.7
P755	(0.04)	0	3/25	Before 5:00	7.79	3.1	8.23	3.1
			3/27	Before 11:30	8.70	4.5	8.81	4.2
A193	(0.04)	20	3/26	4:40–5:10	10.31	4.6	10.61	4.6
P889	(0.04)	20	3/27	1:50–8:30	7.48	3.0	7.42	3.7
			3/29	At 9:40	7.91	3.5	7.55	5.0
P887	(0.04)	20	3/27	1:50–8:30	8.87	4.0	8.29	4.1
			3/29	Before 7:50	10.24	4.5	9.62	4.6

TABLE 4—*Concluded*

NUMBER OF BIRD	DOSAGE		DATE OF EGGS	TIME ACTUALLY LAID (1STS = A.M.) (2NDS = P.M.)	TREATED EGGS		MEANS FOR CONTROL EGGS	
	Amount	Time before first egg			Weight	Rate of loss	Weight	Rate of loss
	grains	*hours*			*grams*	*mgm.*	*grams*	
P659	(0.02)	44	3/31	Before 4:50	8.21	3.2	8.46	3.2
			4/2	Before 8:25	8.94	3.5	8.87	3.5
907	(0.01)	20	4/2	At 5:37	9.25	4.0	9.31	3.3
			4/4	9:30–9:45	9.93	4.0	9.79	4.9
A798	(0.02)	98	4/2	At 5:56	8.85	3.4	8.95	4.8
			4/4	9:00–9:15	10.17	2.3	11.03	5.7
A843	(0.02)	164	4/5	At 5:10	8.25	3.2	8.17	4.1
			4/7	9:30–10:00	Broken			
P887	(0.02)	284	4/7	4:40–4:50	8.58	3.8	8.29	4.1
			4/9	Before 9:00	9.67	4.3	9.62	4.6
P889	(0.02)	356	4/10	At 4:35	7.09	3.0	7.42	3.7
			4/12	10:00–11:00	8.31	3.6	7.55	5.0
P659	(0.02)	284	4/10	At 4:56	7.84	3.7	8.46	3.2
			4/12	At 7:53	8.53	3.7	8.87	3.5
907	(0.01)	212	4/10	At 5:36	9.04	3.3	9.31	3.3
			4/12	At 8:48	9.79	4.1	9.79	4.9
A798	(0.02)	332	4/12	5:10–6:50	8.74	3.7	8.95	4.8
			4/14	9:30–9:45	9.72	5.7	11.03	5.7

[4] These four birds were dosed (three times daily) with atropine sulfate.

[1] This bird visibly much affected by dosage. First egg in life for no. P853.

[2] Dosage above this point in table was three times daily; including and below this point all dosage was twice daily.

[3] Shell thin and soon crushed.

which it was subjected to atropine. A dosage of 0.04 grain thrice daily brought one of two birds near to collapse, but neither this dosage nor one-half of this amount affected considerably or measurably the amount of shell material secreted by four birds as this was measured by the hourly rate of loss weight in table 4.

Cocaine. The secretion of albumen (last two birds of table 2) under cocaine was probably slightly decreased. The amount of shell material

apparently decreased by about 5 per cent. The percentage of bases present in the shell ash was little if at all affected; possibly it was slightly decreased but there is even less evidence of this than there was in the case of atropine.

Nicotine. Twelve eggs were obtained from four birds treated with nicotine (table 1, and first three birds of table 2). In only about one-half of these was the nicotine given before the secretion of albumen was completed. This fact and other special circumstances concerned in the figures obtained make it doubtful whether the data show an

TABLE 5

Analyses of albumen secreted under atropine dosage (0.005 grain, twice daily) and control

	NUMBER OF ANALYSIS	WEIGHT	PERCENTAGE (MOIST WEIGHT)		
			Alcohol insoluble	Alcohol soluble	H_2O
		grams			
Control	1	6.341	7.67	1.12	91.21
	2	6.417	8.37	1.26	90.37
	3	6.438	8.28	1.06	90.66
	4	6.692	7.12	1.08	91.80
	5	5.161	7.20	1.26	91.54
	6	6.340	7.39	1.10	91.51
	Average...	*6.232*	*7.67*	*1.15*	*91.18*
Atropine	7	6.204	8.01	1.25	90.74
	8	6.493	7.89	1.14	90.97
	9	6.371	8.28	1.22	90.50
	10	6.078	7.94	1.23	90.83
	11	5.694	8.28	1.10	90.62
	12	6.209	7.45	1.29	91.26
	Average....	*6.175*	*7.98*	*1.21*	*90.82*

effect, or indeed whether adequate opportunity was offered to produce an effect, on the secretion of albumen. The data for effects upon the total shell material are conflicting and of uncertain meaning. The nicotinized eggs obtained from no. A120 (table 1) were the smallest group of the series. Nevertheless the average amount of shell placed upon these eggs was absolutely the largest in amount. For no. A843 (table 2) also the relative proportion of shell material is probably high. For eggs derived from the other two birds (nos. 907, A798, table 2) the amount of shell material is less than normal. The proportion of bases found in this shell material was quite normal.

The data seem to indicate that about one-sixth of the lethal dose of nicotine, given either once or twice daily, neither notably nor wholly consistently affects the amount or gross nature of the shell material secreted.

Pilocarpine. The shells of eggs derived under dosage with pilocarpine were not removed, weighed and analyzed. These eggs were given careful incubation under other birds and the relative thickness or adequacy of their shells estimated by the rate at which such eggs lost weight, i.e., the rate at which the shells permitted the passage of water vapor from the eggs. Previous work (5) has shown that the thinner the shell the more rapid is the rate of loss of weight by the egg, and one purpose of the present study was to further the ultimate discovery of a means of preventing the formation of shells whose rate of loss of water is so high as to be incompatible with complete embryonic development in the egg. In table 4 this rate of loss from treated eggs may be compared with the mean for two corresponding (first or second of clutch) control eggs produced by the same bird immediately before and immediately after the treated eggs.

The table permits the following comparisons concerning eggs whose shells were produced under pilocarpine dosage:

13 (first of clutch) gave a rate of loss of 3.6, against 3.9 for control.
15 (second of clutch) gave a rate of loss of 4.2, against 4.8 for control.
14 (first of clutch) had a mean weight of 8.46, against 8.60 for control.
17 (second of clutch) had a mean weight of 9.14, against 9.16 for control.

Of these 28 eggs which permit comparisons of relative rate of loss of treated and control eggs, 17 treated eggs show a lower rate of loss; 6 treated eggs a higher rate of loss; 5 show equality of rate of loss. Since the treated and control eggs are of equal size the reduced rate of loss from this large proportion of shells produced under pilocarpine is almost certainly not the result of chance. The average reduction of the rate of loss for the 28 treated eggs compared with the controls is exactly 10.0 per cent. For the first eggs of the clutch (which were necessarily subjected to a somewhat shorter period of dosage), the reduction was 7.7 per cent; while for second eggs of the clutch this was 12.5 per cent. The data therefore indicate that the secretion of shell material is measurably increased, or made more adequate, under pilocarpine dosage. It is clear, however, that in the case of certain birds (P891, P853) which were producing abnormally thin shells the pilocarpine dosage wholly failed to induce the secretion of a normal amount of shell material.

The birds grouped at the bottom of table 4 were dosed with pilocarpine throughout the final period of yolk growth in addition to the period of albumen and shell secretion. These eggs therefore give some opportunity to determine whether and how pilocarpine influences total egg size. The thirteen eggs thus treated average 8.81 grams; the thirteen controls, 9.03 grams. On the other hand, nine eggs treated only during the period of albumen or shell secretion average 8.93, their controls 8.78 grams. It is possible therefore that pilocarpine slightly increases the secretion of albumen but reduces the amount of yolk production. The lack of information concerning the yolk-size of these eggs as well as the limited numbers involved makes a decision on the latter point more or less uncertain. The data supply fairly good evidence, however, that the amount of shell material is somewhat increased under pilocarpine and that the amount of albumen is certainly not decreased, but probably slightly increased when the dosage is limited to the period of actual albumen and shell secretion.

The shells of five eggs shown in table 4 were produced under heavy *atropine* dosage and their adequacy was studied by means of their rate of loss as was done in the case of the shells produced under pilocarpine. The numbers involved are here too few to be of much significance. Three of the atropine-treated shells show a lower rate of loss and two of them a higher rate of loss than their respective controls.

Accurate data concerning the time of egg-laying were obtained throughout the present study. This was necessary in order that one might know whether any increase or decrease of the secretions could be accounted for by a longer or shorter period occupied by the passage of the egg down the oviduct. Originally these data were included in all of the tables, but in order to economize space in already overcrowded tabulations these were ultimately omitted. It is well known that at any given season doves deposit their eggs at a markedly definite hour. Our data show a slightly greater irregularity of time of laying under dosage than under control. In case of none of the drugs used, however, was either an acceleration or a delay of the time of laying clearly or consistently obtained. The measured amounts of the various secretions are therefore uninfluenced from this source; it follows moreover that the movements which propel the egg down the oviduct were either practically unaffected by these drugs or, if such motor responses were present, they nevertheless failed to affect the time of the final expulsion of the egg.

DISCUSSION

In any consideration of the activity of the oviduct it is important that one striking peculiarity of this organ be not overlooked. The oviduct passes repeatedly and periodically from a functional to a semi-functional state within the period of one week or slightly more; and during this period the size of the organ may change by no less than 200 to 400 per cent. The functional organ may occasionally attain perhaps even fifty times its non-functional size. This enormous fluctuation in size may have a bearing upon the innervation—or the lack of it—of the gland cells which form a prominent part of the functional organ. The appearance of the actively secreting glandular cells of the fowl has been briefly described by Cushny (9).

In mammals Loeb (10) was able to show that at a definite period after ovulation the internal secretion of the corpus luteum sensitizes the mucosa of the uterus in such a way as to enable it to form the maternal placenta in response to a contact stimulus provided by the ovum. An essentially parallel situation in birds would involve: First, the preparation of the oviduct for its functional state (growth) under the influence of an internal secretion derived from the ovary; second, the active production of albumen and shell (secretion) in such a prepared oviduct in simple response to the contact stimulus supplied by the passing ovum or other foreign body. The meager modification of the amount of albumen and shell secretion obtained by us by means of drugs with presumably pronounced action upon the nerve supply of the oviduct thus affords some evidence that this hypothetical mechanism also best coincides with the facts; and, further, that this mechanism is little if at all directly affected by nervous action.

Several studies with the same drugs used by us have been made upon the motor nerves of the uterus of various mammals but for reasons stated at the beginning of this paper we do not feel in a position to undertake a discussion of the relation of that work to the present study. However, the rather special relation of pilocarpine and atropine to uterine movements in the cat and the rabbit should be noted here. Cushny (11) says of atropine and pilocarpine: "These two drugs seem to affect some structure, which is different from that acted upon by the other drugs examined (nicotine, ergot, adrenalin, quinine), and which does not appear to be involved in the spontaneous contraction and the response to nerve stimulation." Our data indicate a more or less similar action of atropine, cocaine and nicotine, and an opposed action

of pilocarpine, on the oviducal secretions of the bird. In an earlier investigation Riddle and Anderson (12) found, in the same sort of birds used in the present study, that quinine very markedly decreases the secretion of albumen and of egg yolk. They were inclined to interpret this as a result of the nitrogen-conserving action of the drug. It is possible that the small effects obtained with the drugs used in the present study are also due to effects on the general metabolism of the birds; and that, just as atropine and pilocarpine are not supposed to affect lymph formation in mammals, they have also no direct action upon the oviducal secretions of birds. The effect of quinine on shell secretion was not quantitatively studied in the investigation cited above but it is almost certain that no pronounced change was effected.

If atropine retards and pilocarpine accelerates development within relatively undifferentiated cells, as found by Mathews (13) in dividing starfish and sea urchin eggs, it is also possible to consider the rather small observed effects of these drugs upon the oviducal secretions described here as due to a direct action of the drugs upon the secreting cells.

SUMMARY

Atropine administered twice daily and in the dosages used by us decreases the amount of the output of the albumen-secreting glands but the decrease is only about 2 or 3 per cent.

The albumen secreted under atropine is of normal composition with reference to its water content and the relative proportions of alcohol-soluble and alcohol-insoluble constituents.

The amount of shell material secreted under atropine is not measurably affected. The percentage of bases present in the shell ash is unaffected or only slightly reduced.

Cocaine probably decreases the output of the albumen-secreting glands. The amount of shell material was apparently decreased by about 5 per cent under cocaine. The percentage of bases present in the shell ash was not decisively affected.

Nicotine given once and twice daily did not affect, in any constant or definite way, the amount or the gross nature of the shell material secreted. Its effect upon albumen secretion was not adequately tested.

Pilocarpine probably slightly increases the secretion of shell material and, when the time of dosage is properly restricted, of albumen also.

The results indicate that the occasional imperfect functionings of the avian oviduct which result in the production of inadequate egg

shells probably cannot be even temporarily corrected by means of alkaloidal drugs.

The nature of the innervation of the oviducal glands of birds is apparently unknown. Whether the drugs used in this study act upon the autonomic nerves of birds in a way parallel to their action in mammals is very inadequately known. Definite conclusions are not drawn by us as to the relation of effects produced by these drugs to the nature and extent of the innervation of the oviduct.

If the innervation of the oviduct is similar to that of the mammalian uterus, and the alkaloids used by us have an action on the autonomic nerves of birds similar to their action in mammals, these results supply some evidence that the oviducal secretions of birds occur largely independently of the nervous system. The small effects observed are possibly ascribable to the direct action of the drugs on the secreting cells, or to more general action on the metabolism of the animal.

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COMPARATIVE STUDIES OF THE EARLY REACTIONS IN SPINAL CATS PRODUCED BY VARIOUS METHODS

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Received for publication June 6, 1921

The unpredictability of shock after spinal transection, in cats, by various methods, stimulated this investigation of the possible relation of the early reactions in spinal cats to the surgical treatment to which such animals are subjected.

Experiments recorded in the literature were performed on various animals for the purpose of observing reflex phenomena over long periods of time The operative procedures were conducted with the animals in surgical anesthesia, which precluded early reflex responses after the spinal cord was transected. Early, frequent and accurate blood pressure determinations were not made.

This investigation was conducted upon cats under very light anesthesia at the time of the spinal transection in order to study reflex phenomena and other reactions, instantly after cutting the cord.

METHOD: *Procedures common to all series.* The animals were completely anesthetized with ether from a cone. The blood pressure was taken at intervals throughout the experiments from the left femoral artery, instead of either carotid, to prevent interference of the cannula with subsequent operation in the neck.

The ether was discontinued, and after anesthesia became very light, the cord was cut.

Transection was effected as quickly as possible to preclude hemorrhage.

Reactions to the following stimuli were observed, immediately after the spinal cord was severed, and from time to time throughout the experiment: *a*, sudden pinching of the toes of the hind feet, *b*, pinching of the skin of the hind legs and abdomen, *c*, constant and intermittent pressure against the plantar surface of the hind feet, *d*, scratch irritation of the girdle, *e*, suspension of the animal by the skin of its back,

f, pinching of different portions of the tail as a whole, and of various skin areas of the same, *g*, pinching of the testes singly and together, *h*, striking the various tendons from the pelvis to the toes, inclusive.

The incidence, duration and type of reflex response were carefully observed after the cord was severed.

The length of time the animals lived, after the transection, was also observed.

The spinal canal was carefully opened post mortem, to determine the success and location of the transection.

Further procedures differed in the three series.

Series I. The right vertebral and carotid arteries were ligated, and followed by ligation of the left vertebral and carotid arteries. The cats were tracheotomized and given artificial respiration. Transection of the spinal cord was done in the region of the first cervical segment.

Series II. The cats were given artificial respiration, and the cord was severed in the region of the first cervical segment.

Series III. Without either ligation of the vertebral and carotid arteries or artificial respiration, the spinal cord was divided between the second and third dorsal segments.

Results: *Series I. Blood pressure variations.* The initial blood pressure in this series of twelve animals averaged 153 mm. Hg. (table 1).

The blood pressure in nine of the animals was depressed, on an average, 31 mm. Hg. (from 2 to 66 mm. Hg.) following ligation of the right vertebral and carotid arteries. This represents a percentage fall from the original average blood pressure of 20.9 per cent. Blood pressure in one animal was not determined at this period. The remaining two animals, however, showed increases of 17 to 28 mm. Hg., respectively.

When in addition to ligation of the right vertebral and carotid arteries, the left vertebral and carotid arteries were ligated, the blood pressure rose on the average of 17 mm. above the initial pressure. On the whole, therefore, ligation of the right vertebral and carotid arteries lowered the blood pressure below the original level, and subsequent ligation of the left vertebral and carotid arteries raised the pressure, not only to the initial pressure level, but above it slightly.

Immediately after completion of these ligations artificial respiration, by tracheal insufflation, was instituted. This procedure while slightly reducing the blood pressure did not depress it below the initial level.

Transection of the spinal cord in the region of the first cervical segment followed. After a lapse of 2 minutes, on the average, the blood

pressure became constant on a new but lower level. The average level following this procedure was 67.5 mm. Hg in contradistinction to a pressure of 153 mm. Hg initially, and 154.9 mm. Hg just before the cord was cut. The blood pressure, therefore, was reduced some 85 mm. Hg or approximately 55 per cent as an immediate result of the transection.

Temperature. The fall of temperature of the body surface in the animals living as long as 15 or 20 minutes after severing the cord was

TABLE I

Series I. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION	PER CENT FALL OF BLOOD PRESSURE IN CATS NOT GIVING REFLEXES IMMEDIATELY AFTER TRANSECTION	PER CENT FALL OF BLOOD PRESSURE IN CATS GIVING REFLEXES IMMEDIATELY AFTER TRANSECTION
no.	*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*	*minutes*	*per cent*	*per cent*
1	184	56	128	69.5	3	69.5	
2	144	84	60	41.6	2	41.6	
3	198	86	112	56.5	3	56.5	
4	142	40	102	71.8	7		71.8
5	144	76	68	47.2	134*		47.2
6	114	64	50	43.7	58		43.7
7	156	130	26	16.6	54*		16.6
8	154	35	119	77.2	120	77.2	
9	136	36	100	73.5	14		73.5
10	178	60	118	66.2	57*	66.2	
11	130	64	66	50.7	44		50.7
12	156	80	76	48.7	5		48.7
Average...	153	67.5	85.4	56.1	28.4†	62.2	50.3

* Experiment interrupted before animal died.

† Average life of those that died.

sufficient to detect, unquestionably, by touching the surface of the animal.

Reflexes. Instantly after the spinal transection, five of the animals showed no muscle tonus or reflex response of any kind (table 2). The remaining seven of the twelve animals responded with at least one type of reflex.

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The relative individual depressions

from the initial pressure in the five animals giving no reflex response ranged from 41.6 per cent to 77.2 per cent (table 1), and those in the seven animals giving reflex response ranged from 16.6 per cent to 73.5 per cent.

Fate of the animals. The duration of life of the animals of this series was, for the most part, short. Of the twelve animals, nine died on an average of 28.4 minutes (varied from 2 to 120 minutes) after the cord was cut. The remaining three were killed at periods of 134, 57 and 54 minutes after the spinal transection.

TABLE 2

Series I. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	TIME OF APPEARANCE OF REFLEXES AFTER TRANSECTION	DURATION OF REFLEXES	DURATION OF LIFE AFTER TRANSECTION	TRANSECTION
no.		*minutes*	*minutes*	*minutes*	
1	None		0	3	Complete
2	None		0	2	Complete
3	None	2	1	3	Complete
4	Reflexes	Immediately	1	7	Complete
5	Reflexes	Immediately	58	134†	Complete
6	Reflexes	Immediately	5	58	Incomplete
7	Reflexes	Immediately	29*	54†	Incomplete
8	None	10	45	120	Complete
9	Reflexes	Immediately	11	14	Complete
10	None	7	48*	57†	Complete
11	Reflexes	Immediately	42	44	Complete
12	Reflexes	Immediately	3	5	Incomplete

* Experiment interrupted at this time, reflexes still active.
† Experiment interrupted before animal died.

Post-mortem examination. Three animals in this series showed almost but not quite complete transection of the cord.

Series II: Blood pressure variations. In this series of six cats the average initial blood pressure was 154.6 mm. Hg. (table 3).

Institution of artificial respiration, by tracheal insufflation, was followed by a decrease of blood pressure to 127.2 mm. Hg.

The spinal cord was then transected in the region of the first cervical segment. Within 2 minutes the average absolute blood pressure was constant at 89.6 mm. Hg. The pressure, therefore, was now 65 mm. below the initial level, which represents a relative depression of 42 per cent.

Temperature. There was a decrease of body surface temperature 15 or 20 minutes after cutting the cord sufficient to detect by palpation.

Reflexes. Each of the six animals gave flexion and extension instantly after transection of the cord (table 4).

TABLE 3

Series II. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION
no.	*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*	*minutes*
1	146	120	26	17.8	11
2	204	90	114	55.8	39
3	190	92	98	51.3	42
4	144	60	84	58.3	20
5	148	142	6	4.0	10
6	96	34	62	64.5	100*
Average.....	154.6	89.6	65	41.9	24.4†

* Experiment interrupted before death occurred.

† Average life of those that died.

TABLE 4

Series II. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	DURATION OF REFLEXES	DURATION OF LIFE AFTER TRANSECTION	TRANSECTION
no.		*minutes*	*minutes*	
1	Reflexes	5	11	Complete
2	Reflexes	37*	39	Complete
3	Reflexes	38	42	Complete
4	Reflexes	19*	20	Complete
5	Reflexes	10	10	Incomplete
6	Reflexes	93–99*	100†	Complete

* Reflexes were present at this time but had been lost for a part of the period of observation.

† Experiment interrupted while reflexes were absent.

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The individual absolute depressions from the initial blood pressure ranged from 6 to 114 mm. Hg, and the individual relative falls ranged from 4 per cent to 64.5 per cent. All of the animals gave reflex response.

Fate of the animals. The duration of life was short in this series, five of the animals dying on an average of 24.2 (varied from 10 to 42 minutes) minutes after the spinal transection. One animal was killed at the expiration of 100 minutes.

Post-mortem examination. One animal of the series showed not quite complete transection of the cord.

Series III: Blood pressure variations. The initial blood pressure in this series of eight animals averaged 173.7 mm. Hg (table 5).

Without further operative procedures, the spinal cord was transected between the second and third dorsal segments.

TABLE 5

Series III. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION
no.	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*minutes*
1	162	156	6	3.7	48 hrs.*
2	178	150	28	15.7	120*
3	168	75	93	55.3	125*
4	190	152	38	20.0	82*
5	154	92	62	40.2	30*
6	164	106	58	35.3	112*
7	194	76	118	60.8	39*
8	180	114	66	36.6	44*
Average.....	173.7	115.1	58.6	33.7	

* Experiment interrupted at this time.

After an average interval of 2 minutes the blood pressure was stabilized at 115.1 mm. Hg. The absolute decrease from the initial level averaged 58 mm. Hg, and the relative depressions averaged 33.7 per cent.

Temperature. Although accurate determinations of the body surface temperature were not made throughout this investigation, the animals of this series did not manifest sufficient temperature variations to detect by feeling the surface of the body.

Reflexes. Each member of this series gave two or more types of reflex response instantly after severing the cord (table 6).

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The individual absolute depressions from the initial blood pressure ranged from 6 to 118 mm. Hg, and the

individual relative depressions from the same level ranged from 3.7 per cent to 60.8 per cent. All of the animals, however, gave reflex response under these various conditions of blood pressure.

Fate of the animals. Not one animal died during the periods of observation, which varied from 34 minutes to 48 hours. The usual period of observation, however, approximated 2 hours.

Post-mortem examination. The spinal cords of three animals of this series were not quite completely severed.

Note: The reactions in those animals undergoing almost but not quite complete transection were so comparable to those of the cats after complete severance of the cord that no attempt has been made to separate the two classes.

TABLE 6

Series III. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	DURATION OF REFLEXES	TRANSECTION
no.		*minutes*	
1	Reflexes	48 hrs.*	Complete
2	Reflexes	120*	Complete
3	Reflexes	125*	Complete
4	Reflexes	82*	Complete
5	Reflexes	30*	Incomplete
6	Reflexes	112*†	Incomplete
7	Reflexes	39*	Incomplete
8	Reflexes	44*	Complete

* Experiment interrupted but reflexes were still active.

† Reflexes were present at this time but had been lost for a part of the period of observation.

SUMMARY AND CONCLUSIONS

1. Ligation of the vertebral and carotid arteries, before severing the spinal cord, does not reduce the amount of blood loss.

2. The blood lost after spinal transection, with or without previous ligation of the vertebral and carotid arteries, is negligible.

3. The blood pressure begins to fall immediately after severing the cord and becomes constant at its new level within 2 minutes.

4. Spinal transection in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, but without ligation of the vertebral and carotid arteries, produces appreciably less depression of blood pressure than spinal transection under the same conditions, with ligation of the vertebral and carotid arteries.

5. Transection of the spinal cord between the second and third dorsal segments, under very light ether anesthesia and without ligation of the vertebral and carotid arteries or artificial respiration, produces distinctly less fall of blood pressure than when the spinal transection is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, with or without ligation of the vertebral and carotid arteries.

6. When the cord is transected between the second and third dorsal segments the length of life of the animals indicates no impairment of respiratory function. This is contrary to the findings of Gotch and Horsley (1).

7. There is no relationship, within limits, between spinal shock and blood pressure.

8. The incidence of spinal shock, in cats, is unpredictable, when the spinal transection is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, subsequent to ligation of the vertebral and carotid arteries.

9. When spinal transection, in cats, is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, without ligation of the vertebral and carotid arteries, spinal shock does not occur.

10. Spinal shock does not occur, in cats, when the spinal cord is severed between the second and third dorsal segments, under very light ether anesthesia and without ligation of the vertebral and carotid arteries or artificial respiration.

11. From the standpoint of the absence of spinal shock after spinal transection there can be no doubt that the best preparations are those made without previous ligation of the vertebral and carotid arteries. While the evidence strongly indicates a relationship between spinal shock and the surgical trauma preceding spinal transection, it is felt that the data are inadequate to permit of such a definite conclusion at this time.

12. In general, the length of life after spinal transection is much greater when the cord is severed between the second and third dorsal segments, when no ligations to control hemorrhage are made, and no artificial respiration is given.

13. Body temperature falls appreciably in cats undergoing spinal transection in the region of the first cervical segment.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

X. The Vagus Control of the Esophagus

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Received for publication June 3, 1921

This reinvestigation of the motor control of the esophagus by the vagus nerves was prompted by the unexpected type of control by the vagi of the lung motor mechanism in the amphibia (frogs and salamanders). It was found that the primitive amphibian lung possesses independent or peripheral automatism like the heart and the gut and, as in the case of the heart, the local lung automatism is normally prevented from full development by tonic inhibition via the vagi nerves (6), (20). The lungs are diverticula of the esophagus. The question naturally arises whether in the adult stage of any species of mammals the esophagus, or any region of it, retains the type of local automatism and extrinsic inhibitory control shown by the amphibian lung. This question appears to us of peculiar importance in relation to the phenomena of cardiospasm and spasm of the esophagus in man.

It was pointed out in one of our earlier papers that the type of motor control (exclusively inhibitory) found in the most primitive lung available for study (lung of necturus) is probably an original state rather than a result of differentiation, because in the higher amphibia (frogs), with their more developed lungs, motor nerve fibers are present as part of the extrinsic nerve mechanism, while in the reptilia the local lung automatism is but poorly developed, the extrinsic innervation is of the motor type, the inhibitory type being very subordinate, if not completely lacking (7).

It is also evident that the vertebrate esophagus has, at least in some species, undergone a greater degree of differentiation than the lung motor mechanism. In some species the musculature of the entire esophagus has changed to the striated type, and parallel with this change there is absence of Auerbach's plexus and other local ganglia. In other species this transformation of the primitive esophagus has so

far involved only the upper two thirds of this organ. Obviously these changes in structure are paralleled by changes in type of automatism and motor control, but the latter changes may come about in the absence of change in gross structure, as shown by the amphibian and reptilian lung. When the smooth musculature and local nervous system is retained, as is the case with the cardiac and pyloric sphincters, the degree of deviation from the primitive control is a matter for experimental determination. If the primitive inhibitory control of the cardia predominates in a species, cardiospasm could be induced, not by "vagotonia," as is at present held by many clinicians, but by vagus "hypotonia," that is, failure of the tonic vagus inhibition of the cardia. We have not gone extensively into the clinical literature on cardiospasm, but it is well known that many patients with this malady exhibit other symptoms of impaired inhibitions and Strock (32) quotes Kraus as having shown actual atrophy of the vagi in a case of cardiospasm.

LITERATURE

In 1861 Ravitsch (27) reported that section of both vagi in the frog tends to paralysis of the stomach. Ten years later Goltz (12) showed that in frogs destruction of the medulla by pithing it or killing it chemically with huge doses of curare or as the result of inhalation of chloroform caused a markedly hypertonic condition of the esophagus and stomach together with a great increase in the motility of these structures. This hypertonus of the esophagus and stomach with increased motility occurred also after double vagotomy. Once produced the condition was permanent, relaxation of these organs occurring only when the tissues were dead (acute experiments). Goltz therefore assumed that the vagi carried inhibitory fibers to the esophagus and stomach which were in tonic activity. To his great surprise stimulation of the peripheral vagus trunk did not cause the anticipated inhibition of motility but rather augmented the motility of the esophagus and stomach. Goltz furthermore showed that this hypertonus and increased motility of the stomach could be induced by chemical, mechanical, thermal and electrical stimulation of different parts of the skin and small intestines. Goltz, however, did not consider these effects as types of reflex activity but presumed that the intense stimuli employed paralyzed more or less completely the medullary center from which under normal conditions the tonic inhibitory impulses for the esophagus and stomach arose ("Herabsetzung der Lebensenergie"). It seems to be essentially a reflex inhibition of the tonic activity of the medullary center

quite similar to the reflex inhibition of the inhibitory lung motor center of the frog and other amphibia previously described by us (6), (20). Since it was shown that the vagus on direct stimulation caused an increase in the hypertonus and motility which followed its division, it is possible that the hypertonus and increased motility of the esophagus and stomach which arose on sensory stimulation of cutaneous and visceral nerves was in part due to the reflex stimulation of these motor fibers of the vagus together with central inhibition of the activity of the sympathetic fibers carried to the stomach by the splanchnics.

The original observations of Goltz were confirmed and extended by Contejean (9) in 1892 and by Steinach (30) in 1898. Relying on direct observations, Steinach found that on destruction of the medulla or section of the vagi nerves in the frog (R. esculenta), the esophagus passes at once into permanent hypertonus, the stomach and the upper end of the small intestine show increased spontaneous motility and increased excitability to direct stimulation as well as to stimulation of the upper spinal nerves. Steinach varied the experiments by local etherization of the medulla. In this way Steinach found that the hypertonicity and hypermotility of the upper end of the alimentary tract can be induced two or three times in the same animal and the normal conditions restored by removing the ether from the medulla by the aid of irrigation with physiological salt solution. Steinach states that after the third or fourth period of etherization the medulla cannot be restored to normal. Steinach was less successful in causing inhibition of the gastric and esophageal tonus by electrical stimulation of the medulla, but as he relied on direct observation, slight tonus changes following stimulation of the medulla could not be detected. Steinach concluded that in the frog the vagi contain inhibitory fibers for the esophagus, the stomach, and the upper end of the small intestines, and that these fibers are in tonic activity, like the vagus inhibitory nerves to the heart.

Bottazzi (3) reported motor effects but no distinct inhibition of the esophagus of the toad on stimulation of the vagi and the sympathetic nerves. On stimulation of the pure vagi the contraction of the longitudinal musculature predominated.

Stiles (31) showed that isolated segments of the frog's esophagus exhibit a greater degree of spontaneous automatism than similar segments from other regions of the alimentary tract. The reactions of these esophagus segments (circular fibers) to solutions of electrolytes parallel those of the heart.

Waddell (33) found that pituitrin has a primary depressant action on the frog's esophagus, the depression appearing both in the longitudinal and the circular musculature. It is most marked in the cardiac end of the esophagus.

In 1911 Hopf (13) reported extensive experiments on the effect of vagi stimulation on the stomach and esophagus of the frog, concluding that the vagi contain both inhibitory and motor fibers to the esophagus and stomach, the motor fibers predominating. Hopf stated also that if the esophagus and cardia are put on too great tension the inhibitory action of the vagi on these structures cannot be demonstrated. Unfortunately the experimental procedure followed by Hopf is open to objection, and the numerous tracings published are far from convincing in support of his conclusions.

Patterson (26) found that section of both vagi (vago-sympathetic) in the bull frog rendered the stomach hypertonic for a period of 10 to 15 days. The condition of the esophagus and cardia was not noted.

There appears to be nothing in the literature on the effects of vagus section on the esophagus and cardia in the reptilia. Bercovitz and Rogers (1) have recently shown that section of the vagi in the turtle may increase the gastric tonus, at least temporarily, and stimulation of the peripheral vagus with weak induction shocks repeated at slow rate inhibit both gastric tonus and gastric peristalsis, while stronger stimulation of the vagi induces gastric contractions. This indicates that in the turtle the vagi contain both motor and inhibitory fibers to the stomach.

Doyon (10) reported that appropriate stimulation of the peripheral vagus in the pigeon inhibits the motility of the gizzard, and that this inhibitory action is abolished by nicotin.

Observations on mammals are more numerous. Bernard (2) and Schiff (28) reported a temporary (10 to 72 hours) spasm of the esophagus and cardia following section of the vagi in dogs. Chauveau (8) noted a similar spasm of the esophagus in three horses following double vagotomy. In all horses double vagotomy was followed by paralysis of the esophagus.

Kronecker and Meltzer (17) reported reflex dilatation of the cardia, but ascribed this to central inhibition of the vagus motor tonus to the cardia. Later (1906) Meltzer and Auer (23) showed that the vagus contains inhibitory fibers to the cardia of the rabbit and that these can be excited reflexly by stimulation of the central end of the remaining vagus.

Openchowski (24), (25) described in the rabbit, dog and cat a branch of the vagus on the lower end of the esophagus, whose stimulation caused dilatation of the cardia. This nerve was accordingly called "nervus dilator cardia." The dilatation of the cardia was also obtained by stimulation of the peripheral end of the vagus with weak induction shocks of slow rhythm. With stronger shocks at a rapid rate contraction of the cardia was induced.

This investigator also describes groups of ganglion cells, in addition to the plexus of Auerbach, in the region of the cardia. These nerve cell groups are similar in structure to those of the heart ganglia. He concludes further, on the basis of experiments on rabbits, dogs and cats, that there is a constrictor center for the cardia in the posterior corpora quadrigemina, while the primary dilator center for the cardia is located in the region of the union of the nucleus caudatus and nucleus lenticularis. Most of the constrictor fibers for the cardia pass out in the vagi, principally the left, but a few reach the cardia via the cervical nerves and the cervical sympathetic. Some of the dilator fibers for the cardia also pass out through the cervical nerve roots and reach the cardia via the aortic plexus.

Openchowski states that, with the vagi intact, reflex dilatation of the cardia may be induced by stimulation of the kidneys, the uterus, the urinary bladder, the intestines or the sciatic nerve. Dilatation of the cardia is sometimes induced by stimulation of the cerebral cortex in the region of the crucial sulcus.

Langley (18), working with curarized and atropinized rabbits, reported that stimulation of the peripheral vagus caused dilatation of the cardia. When no atropin was administered the vagus stimulation caused increased tonus of the cardia. Langley therefore concluded that the vagus nerves carry both motor and inhibitory fibers to the cardia, the motor fibers being more readily paralyzed by atropin. Later Langley (19) reported that the adrenalin inhibits the cardiac sphincter in the rabbit.

Krehl (16) reported that section of both vagi in the dog left the esophagus and the cardia atonic or patulous. The state of the cardia seems to have been determined by the absence of resistance to the passage of a stomach tube into the esophagus through a gastrostomy. These findings of Krehl on the dog are accepted, without additional proofs, by Katschowsky (14), working in Pawlow's laboratory.

On the basis of the examination of four dogs, Sinnhuber (29) reached the conclusion that section of the vagi above but close to the diaphragm

renders the cardia atonic, while section of the vagi high up in the neck leads to a temporary hypertonicity of the cardia. If these observations should prove to be correct, it would seem that in the dog the inhibitory fibers to the cardia leave the vagi at some distance above the diaphragm and pass down to the cardia in the wall of the esophagus.

Espezel (11) declared (in 1901) that inhibitory nerve fibers to the esophagus had not yet been satisfactorily demonstrated, at least for the rabbit and the dog.

Strock (32), working on dogs, reported that section of both vagi causes dilatation of the esophagus, and a slight increase in the tonus of the cardia. He quotes V. Mickulicz to the effect that section of the vagi doubles the tonus of the esophagus as measured by the resistance to the forcing of liquids through into the stomach. Nevertheless Strock does not believe that this hypertonus of the cardia in dogs is analogous to clinical cardiospasm.

Kelling 15) states that deep anesthesia increases the resistance of the cardia to pressure exerted from the stomach side. If we assume that the anesthetic paralyzes the medullary vagus center before paralysis of the local motor mechanism of the cardia, one may conclude that the cardiospasm induced by deep anesthesia is caused by elimination of the vagi inhibitory impulses to the cardia.

Cannon (4) reported (1907) that section of both vagi in the cat leads to an increased tonus of the cardia lasting for several days (and in some cases indefinitely), parallel with decreased tonus and peristalsis of the esophagus. This conclusion was based on the observation that the peristalsis of the lower esophagus frequently failed to force the food through the cardia, and the increased resistance at the cardia to the passage of the stomach tube.

The resistance of the cardia to the passage of food may not indicate any increased tonus of the cardia, but merely the failure or absence of the normal inhibitory reflex. We do not believe that under normal conditions the cardia is forced mechanically by the strength of the esophagus peristalsis. It seems more probable that the passage of food through the cardia in normal swallowing is associated with reflex inhibition of the tonus of the cardia. Furthermore, the degree of resistance offered to the passage of the stomach tube is subject to many factors, so that actual quantitative differences, even if established, are capable of more than one interpretation.

EXPERIMENTAL METHODS

Turtles. In practically every case the animals were decerebrated as a preliminary step, to avoid the use of anesthetics in subsequent operations. In a few experiments the spinal cord was sectioned below the medulla, leaving the brain intact. Since all subsequent dissection is made peripheral to the spinal transection, no pain impulses can reach the brain.

The animal is secured, ventral side down, on the turtle stand, in a manner described in a previous communication (7). The entire spinal cord is pithed, the spinal column of the neck and the large retractor neck muscles excised. We usually made a window through the carapace on the left side, exposing the left lung and the stomach. The left lung was prepared for the administration of artificial respiration.

Three methods of introducing and adjusting the delicate balloons in the esophagus and the stomach were tried out. Through a small slit in the stomach 2 cm. from the cardia a flexible seeker was passed through the esophagus; the gastric and esophageal balloons were attached to this seeker and pulled back into their respective positions, the flexible rubber tubes connecting the balloons with the respective water manometers. This method has the disadvantages of the trauma to the stomach wall, the mechanical action on the rubber tubes of the swallowing or respiratory movements of the jaws, and possible reflex influence on the esophagus and stomach (via the medulla) from the irritation of the tubes in the mouth and pharynx. To avoid these latter factors we made a small opening in the wall of the esophagus just below the pharynx and passed the tubes from the manometers through this slit, thus leaving the mouth free from mechanical stimulation.

In a few experiments the stomach was left intact, the balloons being pulled through the esophagus and stomach into their proper positions through a slit in the duodenum near the pylorus. We also pushed the balloons into position by means of a seeker operated through the esophagus. This leaves the stomach intact. When records were taken from the esophagus only, the balloon was either pushed into position by means of a seeker operated through the mouth or pulled into place from an opening into the stomach.

The tubes connecting the balloons must be anchored at the head end to prevent them from being pushed from the esophagus into the stomach, and from the stomach into the intestine by the esophageal and gastric peristalses. Since by our methods of preparation both esophagus and

stomach are exposed from the dorsal side, moderate inflation of the balloons will disclose their exact location by direct inspection. Furthermore, the exact location of the respective balloons was verified by opening the esophagus and stomach at the end of each experiment.

A few observations were made on the action of the vagus nerves on the esophagus, after removal of the esophagus from the body. The excised esophagus was kept moist with Ringer's solution but no attempt was made to perfuse the organ.

It is scarcely necessary to state that there is considerable hemorrhage in the turtles as a result of the extensive dissection required to remove all external influences on the upper end of the gut. There was accordingly considerable impairment of the circulation. Impaired circulation in the esophagus and stomach may not only induce abnormal motor activities, but will be a source of error in a study of the action of drugs, when these are injected intravenously. To control these sources of error the loss of blood was compensated for by intravenous injection of Ringer's solution. One can judge the state of the circulation in our preparations fairly accurately by direct inspection, and in all cases of doubt methylene blue was injected intravenously at the end of the experiments, in order to check up on the efficiency of the circulation in the organs concerned.

The pithing of the brain (medulla) was usually made from the spinal cord end. The vagi sections were at a distance of 0.5 to 1.0 cm. from the angle of the jaw. The pulmonary vagi were usually sectioned so that the lung contractions caused by stimulation of the vagi may not influence the tension in the stomach and esophagus.

The esophageal and gastric balloons were made out of rubber condoms, the length of the balloons varying with the size of the animal. The initial pressure in the balloons was usualy fixed at 1 to 2 cm. of water, the graphic registration being in every case made by means water manometers (diameter, 8-10 mm.).

Frogs. The animals were decerebrated, the spinal cord sectioned in the second cervical segment and pithed posteriorly. The animal is thus immobilized, except for the jaws and the pharynx, and there is no call for anesthesia.

In further preparation the animal was fixed, dorsal side down, and an incision made in the median line from the symphysis pubis to the symphysis of the lower jaws, the body wall pinned to the side, and the esophagus and stomach exposed by pushing the left lung and the liver toward the right side. In some cases the left lung was excised after ligation at the base.

Vigorous animals will usually carry on fairly efficient respiratory movements even after this extensive dissection. But we usually placed a cannula in the tip of the right lung for the purpose of artificial respiration when required.

The necessary dissection causes, of course, hemorrhage in varying amounts, and consequent impairment of the circulation. This was counteracted, so far as possible, by introducing Ringer's solution through the median abdominal vein.

With frogs fixed dorsal side down pithing of the medulla from the spinal cord is not feasible. Hence in the experiments involving pithing of the medulla we usually (after the decerebration) cut off the upper mandible at the level of the anterior end of the brain cavity, so that the pithing needle could be readily introduced and pushed down to the medulla with minimum mechanical disturbance of the graphic registrations. In a few experiments we destroyed the medulla by crushing the skull by strong artery forceps, but the procedure cannot be carried out without some direct mechanical stimulation of the pharynx. In several of the experiments the medulla was destroyed by injecting into it through the anterior end of the cranial cavity one drop of chloroform. This method has the advantage of the sudden destruction of medulla without the movements of the head and neck which always occur during pithing it. It furthermore does not bring about a change in the level of graphic registration as does placing the jaws of the hemostat prior to crushing of the medulla.

In animals thus prepared, the effects on the esophagus by pithing the medulla, sectioning the cervical sympathetic and the vagus nerves, and stimulation of the vago-sympathetic nerves, were studied both by direct inspection and by graphic methods. The latter consisted in placing a hook, connected by a recording lever, under the cardiac end of the esophagus, the stomach being left intact, the pharyngeal end of the esophagus serving as the fixed point. We also sectioned the stomach just below the cardia, fixed a hook in the small stomach segment and suspended the esophagus by the weight of the recording lever, the pharyngeal end again serving as the fixed point. But finding that section of the stomach near the cardia induced motor disturbances in the esophagus, we endeavored to eliminate this by suspending both stomach and esophagus by a hook in the duodenum close to the pylorus. Such records are composites of gastric and esophageal motility. These methods of suspension interfere more or less seriously with the circulation in the esophagus and stomach. But any method of graphic regis-

tration of esophageal motility in our preparations introduces serious errors from the movements of the pharynx. These cannot be eliminated, if the medulla is to be kept in functional activity. The data secured by these graphic methods must therefore be checked against those from direct observation. The frog's esophagus is too short to permit recording tonus and motility by the balloon method.

An aspiration bottle containing Ringer's solution was so arranged as to deliver its contents drop by drop to the surface of the stomach and esophagus. In this way we prevented these structures from drying. In those experiments where the action of drugs was studied the irrigation of the esophagus with Ringer's solution was stopped while irrigating the preparation drop by drop with the drug contained in a hypodermic syringe.

The graphic method employed by us introduces an additional disturbing factor, namely, the local or direct stimulating action of tension on the stomach and esophagus, and the failure to record satisfactorily the tonus and contractions of the circular musculature. In order to eliminate the direct stimulating action of tension on the stomach and esophagus, we allowed these structures to remain in their normal position in the abdominal cavity gently hooking back only such structures which hid them from complete view. We next mounted above the preparation a camera. This latter was essentially a Woelfel artificial eye used ordinarily for classroom instruction in physiological optics. We replaced the stationary ground glass "retina" with larger pieces of ground glass which could be readily moved laterally. The ground surface of the glass was uppermost. The light from two ordinary head lights with reflectors was directed toward the preparation. The room was now darkened and the observer, with head and shoulders under a black cloth, looking at the ground glass surface could, by a little focusing, obtain a distinct and slightly enlarged image of the stomach and esophagus. The outlines of these organs were traced on the ground glass with a soft pencil. As soon as this simple sketch was complete the glass was shifted laterally and another sketch was made. After several sketches were made in this manner the vagi were ligated in the neck, and the stomach and esophagus were sketched many times in rapid succession. In this manner we obtained tracings of the outlines of the esophagus and stomach similar to those obtained by Cannon when studying esophageal, gastric and intestinal movements in mammals over x-rays and under a fluoroscopic screen. It took less than 5 seconds to complete a single sketch. These sketches were subsequently traced

on paper by placing tissue paper over the ground glass plate and illuminating the latter from below with desk lamp.

In the observations on drug actions, the drugs (in Ringer's solution) were applied to the surface of the esophagus; injected intravenously; or injected in large doses hypodermically at different times before the preparation of the animals.

It is of some importance that all solutions used for intravenous injections or superficial irrigation have the same temperature as the frog (approximately room temperature); for heat accelerates and cold depresses the peripheral automatic rhythm seen after ligation of the vagus, destruction of the medulla, or stimulation of the peripheral end of the vagus nerve.

RESULTS ON TURTLES

Effects of pithing the medulla or sectioning of the vagi nerves. If the circulation is maintained in good or fair condition the animal, with only medulla, midbrain and vagi intact, usually executes periodic respiratory (swallowing) movements for hours. These swallowing movements of the jaws are accompanied by contraction of the striated muscles of the pharynx. The contractions of these muscles together with the air pressure in pharynx and upper end of the esophagus cause rapid increase in the pressure in the esophageal balloon, shown in figure 1, *R*. But the respiratory act or periods of swallowing appear to be accompanied by actual decrease in the tonus of the esophagus, and the tonus of the esophagus increases gradually between the swallowing periods (fig.1). In the case of preparations that exhibit no spontaneous respiratory or swallowing movements there is usually no distinct rhythm of the tonus of the esophagus so long as the vagi and the medulla are intact.

The pithing of the medulla or sectioning of the vagi close to their exit from the skull induces a very brief inhibition of the tonus of the esophagus, followed by a permenent hypertonus. Typical records showing these reactions are reproduced in figures 1, *B*, and 2. In some preparations the hypertonus of the esophagus following isolation from the medulla exhibited a slow rhythm (fig. 2, *B*). We were unable to determine whether or not this tonus rhythm was peristaltic contractions of the esophagus. But the hypertonus was rarely equal in degree throughout the entire length of the organ. In other words, isolation of the esophagus from the central nervous system not only induces a general hypertonus, but permits also development of local spasms of the esophagus, lasting in many cases throughout the experiment (6 to 36 hours).

Fig. 1. Turtle decerebrated. Records from the stomach, s, and esophagus e; x, pithing of the medulla; r, swallowing movements (respiration). A, showing tonus rhythm of the esophagus inhibited by the swallowing acts; B, hypertonus of the esophagus and initiation of gastric contractions by pithing of the medulla.

Fig. 2. Turtle decerebrated. Record of tonus of the esophagus. A, x, section of both vagi near the head. B, x, pithing of the medulla. Showing permanent hypertonus and tonus rhythm, B, of the esophagus isolated from the brain.

In preparations also provided with a balloon in the stomach we sometimes observed that destruction of the medulla or section of the vagi induced a rhythm in the stomach (fig. 1, *B*). This confirms the recent observations of Bercovitz and Rogers. It should be noted, however, that pithing of the medulla does not invariably augment the tonus in the active or induce a rhythm in the quiescent stomach, but the hypertonus of the esophagus is always induced by this operation, provided the preparation or the animal is not moribund.

The hypertonus of the esophagus shown in figures 1 and 2 seems to involve mainly the circular musculature. There is no distinct shortening of the esophagus. The esophageal hypertonus seemed to extend to the cardia, but we cannot prove this graphically, as it is not possible to confine a balloon in the cardia without it being influenced by the tonus and contractions both of the gastric and the esophageal musculature.

We desire to point out the similarity of the hypertonus of isolated esophagus of the turtle to the hypertonus and tetanus of the amphibian lung following the same operation, that is, sectioning of the vagi or pithing the medulla

Effects of stimulation of the peripheral vagi. Stimulation of the peripheral vagus (right or left) inhibits the tonus of the esophagus. In preparations in good condition all types and strengths of stimulation applied to vagus nerves cause inhibition so that on the basis of results secured by direct stimulation of the vagus nerves one might conclude that these nerves carry only inhibitory fibers to the circular musculature of the esophagus and cardia. The recovery of the original tonus following the vagus stimulation is usually very gradual (5 to 15 minutes).

Typical tracings illustrating this inhibition are reproduced in figures 3 and 4. In these experiments simultaneous tracings were taken from esophagus and stomach. Tetanization of the vagi with very weak current inhibits the esophagus and may inhibit the stomach (fig. 3). Stronger tetanization of the vagus causes contraction of the stomach parallel with marked inhibition of the esophagus (fig. 4). It will be seen in figure 4 that the esophagus inhibition lasts much longer than the gastric contraction. The esophagus inhibition can be counteracted by intravenous injection of adrenalin (fig. 4, *x*). The vagus inhibitory fibers to the esophagus retain their activity after administration of this drug while the gastric motor fibers of the vagus system are paralyzed.

The inhibition of the stomach by weak tetanization of the vagi confirms the recent findings of Bercovitz and Rogers.

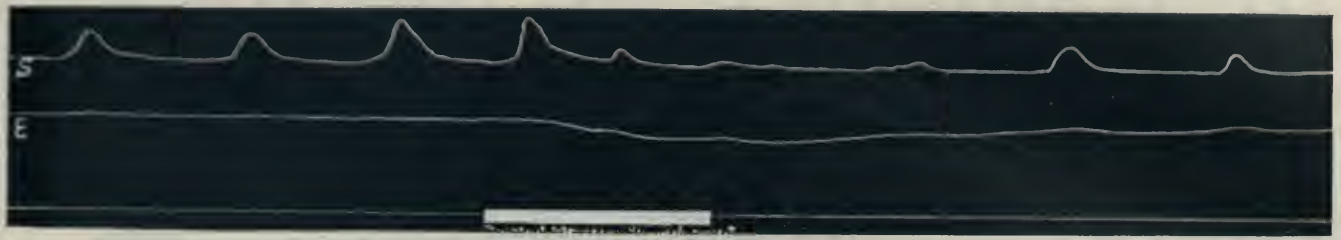

Fig. 3. Turtle, brain pithed. Record of gastric contractions, *s*, and of esophageal tonus, *e*. Signal, tetanization of the left vagus with a very weak current. Showing primary inhibition of the gastric contractions and esophageal tonus by weak stimulation of the vagi.

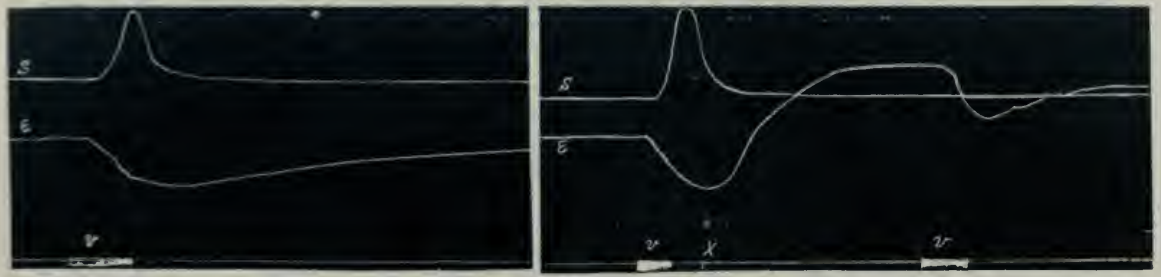

Fig. 4. Turtle, brain pithed. Stomach quiescent, esophagus in hypertonus. Record of gastric contractions, *s*, and esophagus inhibition *e*, on tetanization of the vagi, *v; x*, intravenous injection of 10 cc. 1–100,000 adrenalin, showing stimulation of the esophagus by this drug parallel with the paralysis of the gastric motor fibers of the vagi.

In one preparation we secured what seemed to be primary contraction of the esophagus on tetanization of the esophagus. This animal was in very poor condition, in fact, moribund, at the time of preparation. This reaction was obtained even after removal of the esophagus (with the vagi) from the body so that it is evidently due to contraction in the esophagus itself (fig. 5).

The tetanization of the vagi sufficiently strong to cause contraction of the stomach induces the same contraction of the longitudinal musculature of the lower end of the esophagus ($1\frac{1}{2}$ to 2 cm. above and including the cardia) so that if the esophageal balloon extend down to the cardia, the graphic record represents the algebraic sum of the inhibition of the upper two-thirds of the esophagus (circular musculature) and contrac-

Fig. 5. Turtle. Records from the excised esophagus showing effect of the tetanization of the vagi. *A*, esophagus from an animal in good condition, showing the usual inhibition of esophageal tonus on vagus stimulation. *B*, esophagus from an animal in poor condition (moribund), showing slight increase in tonus from vagus stimulation.

tion of the longitudinal musculature of the lower end of the esophagus, the inhibitory effects predominating.

The results so far show conclusively that the action of the vagi on turtle's esophagus is predominantly, if not exclusively, inhibitory; on the stomach it is predominantly but not exclusively motor. It would thus seem that the hypertonus of the esophagus (circular musculature) following section of the vagi or pithing of the medulla, represents a peripheral automatism normally kept in check by tonic impulses over the vagus nerves, a condition identical with the motor mechanism of the amphibian lung.

We have so far been unable to influence the esophagus by stimulation of the cervical sympathetic nerves, either central or peripheral ends. As in our preparations the entire spinal cord below the medulla was

pithed, no observations could be made on reflex control of the esophagus-vagi inhibitory mechanism, except from the intact cranial nerves. In several preparations with intact medulla and vagi nerves we stimulated the central end of the pulmonary branches of the vagi. This stimulation caused at times dilatation, at times contraction of the esophagus.

3. The action of atropin, nicotin and pilocarpin on the esophagus, and on the vagi fibers to the esophagus. The primary object of these experiments with drugs was to endeavor to separate the inhibitory nervous mechanism from the possible motor nervous mechanism in the manner that proved feasible for the amphibian lung. It will be recalled that in the frog nicotin paralyzes the inhibitory vagi fibers to the lungs, leaving the motor pulmonary vagi functional, so that the pure lung inhibition following vagus stimulation is changed to pure lung contraction after nicotinization. Because of the similarity in origin and neuromuscular structure of the lung and the esophagus, we might look for a similar selective action of nicotin on the vagus system of the esophagus. Support for such selective action is found in the report of Langley on the rabbit that atropin depresses the vagi motor fibers to the cardia to a much greater extent than the inhibitory fibers to that after nicotinization vagus stimulation produces only inhibition of the cardia. Furthermore, Bercovitz and Rogers report on the turtle that atropin paralyzes the gastric motor system of the vagus but not the gastric inhibitory system.

All our experiments with drugs were made on preparations after section of both vagi, or pithing of the medulla, so as to avoid complications from direct action on or reflex action through the medulla. But this means that the esophagus was invariably hypertonic, the degree of hypertonicity varying in different preparations. In most of the experiments in this group parallel records were taken of the gastric and the esophageal contractions to bring out the antagonistic action of the drugs on these two organs.

Atropin, even in very large doses, does not change the action of the vagi on the esophagus. The inhibitory fibers of the vagi are not paralyzed (fig. 6, *B*; fig. 7, *A*, *B*). Because of the fact that atropin usually causes a marked and lasting depression of the esophageal tonus the vagus stimulation prior to atropinization may cause a relatively greater inhibition. But this is no indication of even partial paralysis of the inhibitory nerve fibers.

Nicotin invariably causes a prolonged depression of the esophageal tonus without subsequent stimulation (fig. 8, *A*, *B*). This inhibitory

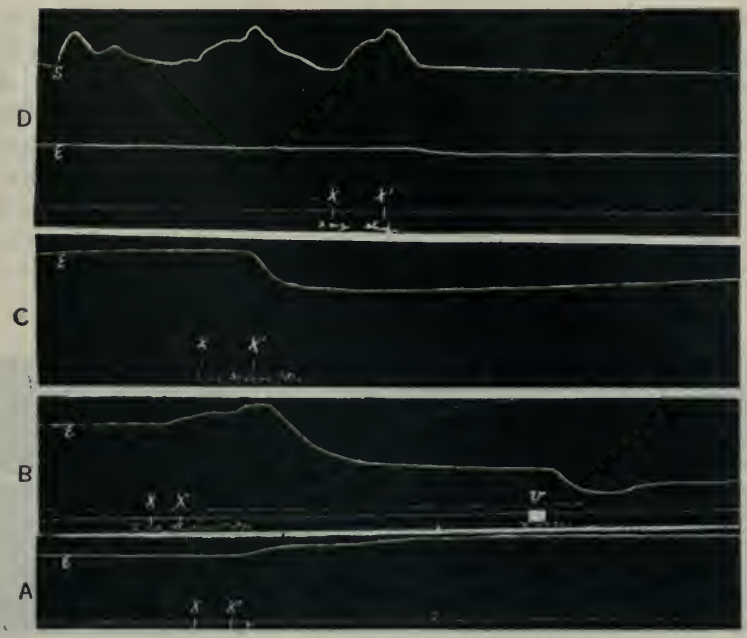

Fig. 6. Turtle, both vagi sectioned. Record of gastric contractions, *s*, and tonus of esophagus, *e*; *x*-*x'*, intravenous injection of atropin sulphate in Ringer's solution. *A*, 1 mgm.; *B*, 2 mgm.; *C*, 1 mgm.; *D*, 2 mgm.; *v*, tetanization of peripheral end of right vagus. Showing primary stimulation, *A*, *B*, and primary inhibition, *C*, *D* of esophageal tonus by atropin; failure of atropin to paralyze the esophageal vagus (inhibitory fibers); primary inhibition of gastric contractions by atropin, *D*.

Fig. 7. Turtle. Record of tonus of the esophagus. Signal, tetanization of peripheral end of vagus (*A*, left vagus; *B*, right vagus). *A*, after intravenous injections of 15 mgm. atropin sulphate and 10 mgm. nicotin. *B*, after intravenous injection of 15 mgm. nicotin. Showing failure of atropin and nicotin to paralyze the vagi inhibitory fibers to the esophagus.

action of nicotin parallels so closely that caused by the stimulation of the vagi as to suggest that nicotin acts primarily by stimulating the local vagus mechanism in the esophagus, as is the case in the heart. But there is no further parallel between the nicotin action on the esophagus and the heart, as this drug fails to paralyze the vagi inhibitory fibers to the esophagus (fig. 7, *B*). We may also point out that nicotin has the same primary action (inhibition) on the amphibian lung as on the reptilian esophagus but this drug tends at the same time to paralyze the inhibitory fibers in the pulmonary vagi, thus bringing the lung in line with the heart.

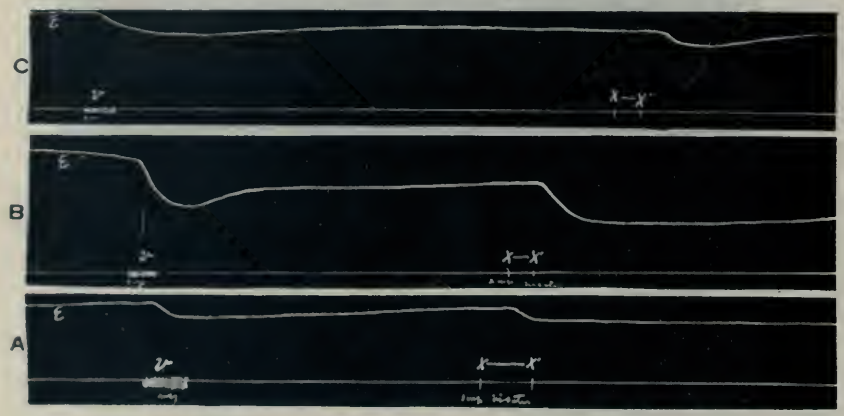

Fig. 8. Turtle, both vagi sectioned. Record of tonus of the esophagus, *e; v*, tetanization of the vagi. *A*, *x-x'*, intravenous injection of 1 mgm. nicotin in 10 cc. Ringer's solution. *B*, *x-x'*, intravenous injection 2 mgm. nicotin. *C*, *x-x*, 10 cc. 1-100,000 histamine hydrochloride. Showing parallel action (inhibition) of vagus, nicotin and histamine.

Nicotin has a primary stimulating action on the turtle's stomach, the stimulation being followed by paralysis. Typical tracings showing the antagonistic action of this drug on the esophagus and the stomach are reproduced in figure 9. The nicotin inhibition of the esophagus is in evidence even after large doses of atropin.

Pilocarpin has a slight inhibitory action on the esophagus, parallel with its stimulating action on the stomach (fig. 10). In no instance did we note any stimulating action of this drug on the esophagus. The stimulation of the motor mechanism of the stomach is the typical action of this drug on the gut, the turtle's esophagus failing to comply with the law.

5. The action of adrenalin and histamine. Adrenalin causes prolonged (30 to 120 minutes) hypertonus of the esophagus (fig. 11). This is true of all concentrations of the drug that are capable of influencing

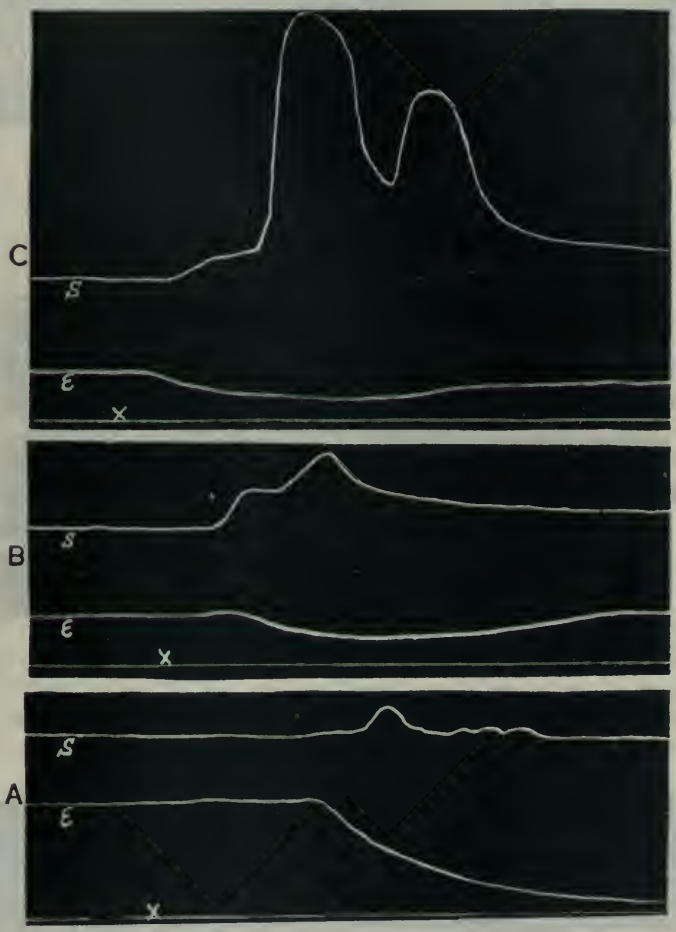

Fig. 9. Turtle, brain pithed. Record of gastric, *s*, and esophageal, *e*, tonus; *x*, intravenous injection of nicotin in 10 cc. Ringer's solution. *A*, 1 mgm. nicotin; *B*, 2 mgm. nicotin; *C*, 2 mgm. nicotin. Showing primary stimulation of the stomach and primary inhibition of the esophagus by nicotin.

this organ. The stimulating action is in evidence even when the esophagus is in marked tonus at the time of intravenous injection of the drug. The action of adrenalin is not influenced by previous adminis-

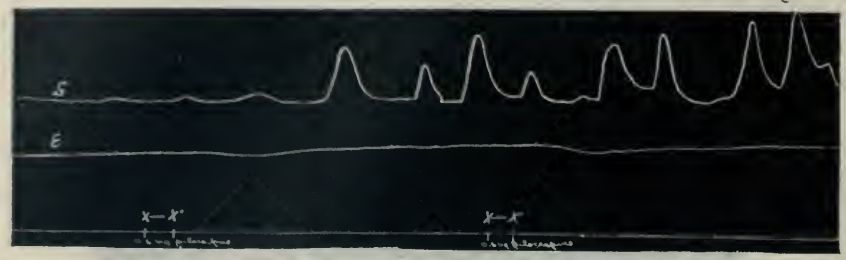

Fig. 10. Turtle. Brain pithed. Parallel record of gastric contractions, s, and esophageal tonus, e; x-x' intravenous injection of. 0.6 mgm. pilocarpin in 10 cc. Ringer's solution. Showing primary inhibition of the esophagus by this drug parallel with the stimulation of the stomach.

Fig. 11. Turtle, brain pithed. Record of tonus of esophagus, E, x, intravenous injection of adrenalin in 10 cc. Ringer solution. A, adrenalin 1–1,000,000; B, 1–500,000; C, 1–100,000. Showing primary and prolonged stimulation of the esophagus by adrenalin.

Fig. 12. Turtle, brain pithed. Record of gastric contractions, s; L, V, tetanization of left vagus; R, V, right vagus; x-x', intravenous injection of 10 cc. 1–100,000 adrenalin in Ringer's solution. There is an interval of 10 minutes between the vagi stimulations at ↑, preparation left for 12 hours. Showing prolonged paralysis of the gastric motor fibers of the vagi by adrenalin.

tration of atropin or nicotin. Adrenalin does not paralyze the vagi inhibitory fibers to the esophagus (fig. 4).

On the turtle's stomach adrenalin has the typical gut action, that is, inhibition. Adrenalin not only depresses gastric automatism and tonus, but it causes a prolonged paralysis of the gastric motor fibers of the vagus system (fig. 12).

The uniform stimulating action of adrenalin on the esophagus was unexpected, in view of the usual inhibition of the gut by this drug, and in view of the predominating, if not sole, *inhibitory innervation* of the esophagus. We have so far failed to influence the esophagus by stimulation of the cervical sympathetic nerve (central and peripheral end). If there are motor fibers to the circular musculature of the turtle's esophagus, they appear to belong to the vagus system, on which adrenalin is not supposed to act. If we accept the usual view that adrenalin action on muscle mechanisms is *ipse facto* evidence of nervous mechanism having similar action our results prove the presence of motor fibers to the esophagus, but the evidence that these motor fibers belong to the vagus sytem is at variance with the sympathomimetic theory.

We have pointed out the parallel between the reptilian esophagus and the turtle lung in the presence of a peripheral local automatism normally held in inhibitory check by the vagi nerves, and the prolonged hypertonus of these organs caused by section of the vagi. But the parallel fails in the case of the primary action of adrenalin which is inhibitory on the amphibian lung and stimulating on the reptilian esophagus.

Histamine is usually regarded as a universal stimulant of smooth muscle. On the turtle's esophagus histamine has a moderate inhibitory action (fig. 8, *c*).

Stimulation of this organ by histamine was never obtained. But the turtle's stomach is stimulated (feebly) by histamine, the turtle's lung is very strongly stimulated. Histamine stimulates the lung musculature of the frog, but inhibits the lung of the salamanders (21), unless the lung inhibitory nervous mechanism is previously paralyzed by nicotin, in which case the histamine inhibition is changed to stimulation (20).

RESULTS ON FROGS

The effect of ligation of the vagus nerves on the motility of the esophagus and cardiac portion of the stomach. If the vagus nerves are ligated in the neck of a frog whose stomach and esophagus are attached by means of a hook and cord to the short arm of a delicate lever recording on the smoked surface of a slowly moving kymograph, the usual and immediate

Fig. 13. Graphic record of the esophageal and, in part, gastric movements of the frog. No decerebration. Spinal cord transected below the medulla and pithed. Animal fastened on its back leaving abdominal viscera exposed. Stomach transected 0.5 cm. from cardia and upper portion attached to delicate recording lever writing on a smoked surface. Lifting ligatures under both vagi. Upper jaw cut off exposing anterior ends of cerebral hemispheres.

A, at *x*, ligation of right vagus; at *y*, ligation of left vagus.

B is continuation of *A*. At *z*, brain pithed with wire through opening in anterior end of cranial cavity.

Indicating an escape of the esophagus and stomach from the tonic inhibitory control of the vagus centers.

effect is a sharp contraction of the longitudinal musculature of the esophagus as is recorded at x in A of fig. 13. Following this initial contraction peristaltic waves pass continuously toward the cardia which may pass over the stomach but usually stop on reaching the cardiac sphincter. These peristaltic waves appearing in the lower end of the esophagus and stomach give rise to the irregular undulation seen in the figure just referred to. The motility then gradually diminishes until the remaining vagus nerve is sectioned as shown at y in A of the figure. Following the physiological division of this nerve from the medullary center the tonus remains lightly above that which existed prior to section of the vagi although this does not obtain invariably in the frogs with which we worked. The subsequent destruction of the medulla at z of B in figure 13 has no appreciable effect in raising or lowering the tone of the esophagus or stomach.

From an examination of this figure it would appear that a division of the vagus nerves relieved, especially the esophagus, from the tonic inhibitory influence of the medullary centers. But results are obtained quite frequently in frogs which would seem to be open to but a single interpretation, namely, that the ligation of the vagus nerves acted solely as a mechanical stimulus to the nerves and that the increased tonus and hypermotility resulting therefrom were due solely to marked after-discharge of motor impulses which, impinging on the ganglion cells of the peripheral automatic mechanisms, continue to send out discharges to the smooth musculature for an appreciable time after the direct stimulation of the nerve fibers in the neck has ceased. This interpretation is rendered quite probable for one of us has shown that the quiescent heart of molluscs can be induced to beat rhythmically for some time following electrical stimulation of the motor heart nerves (5).

Figures 14, 15 and 16, representing essentially the same conditions are offered as evidence in support of this interpretation. In figure 14, A, the right vagus nerve was ligated in the neck with the usual hypertonus and motility of the esophagus. In this animal the medulla had been pithed $1\frac{1}{2}$ hours before taking this record. The response can be accounted for solely on the basis of mechanical stimulation of the motor fibers of the vagus by the tightening of the ligature. In this instance the hypertonus was only transient. Eighty minutes later, electrical stimulation with a tetanizing current (fig. 14,B, at y) induced an activity of the esophagus quite similar to the previous ligation of the vagus in the neck. Figure 15 records a similar experiment and suggests a similar interpretation. It is reproduced solely because the pseudo-hypertonus

on ligation of the vagus (at x in A) and stimulation of the peripheral end (at y in B) records only esophageal movements since the hook attached to the recording lever was placed *under* the lower end of the esophagus.

As a final example of what appears to be a simple mechanical stimulation of the vagus nerve on mechanical division of it in the neck we offer the experiment recorded in figure 16. Here the left vagus was ligated at x in A, with prolonged motor activity of esophagus (and stomach) while in markedly hypertonic state. Simply placing a ligature under the right vagus with the incidental stimulation of the nerve (B at y)

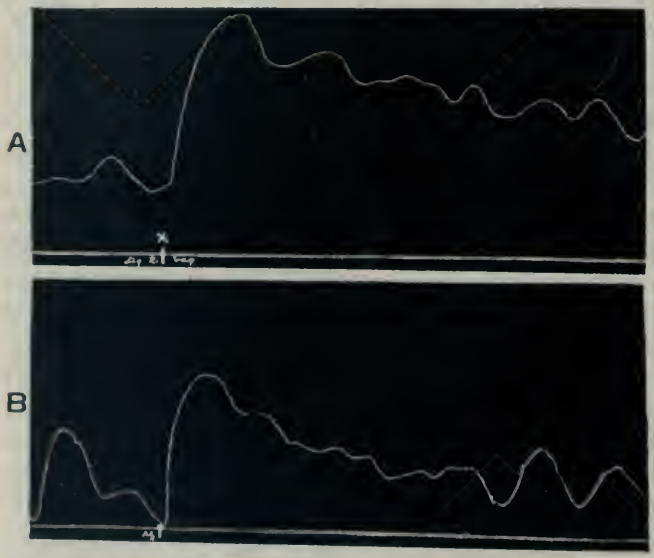

Fig. 14. Graphic record of esophageal movements of a frog. Frog decerebrated, upper jaw cut off exposing anterior ends of cranial cavity. Animal laid on back and virtually eviscerated, the cardiac end of the stomach and entire esophagus alone preserved. The cardiac end of stomach attached to delicate lever. Medulla pithed without striking effects on motility of esophagus. A, record taken 2 hours after beginning of experiment, and about 1½ hour after destruction of the medulla. At x, ligation of the right vagus in the neck. B, at y, electrical stimulation of the right vagus for 2 seconds with a tetanizing current of moderate strength 80 minutes after ligation of the nerve in A. Indicating that the hypertonus of the esophagus following ligation of the vagi in the neck under conditions where these nerves are still in communication with the vagus center is due at least in part to prolonged stimulation of the peripheral automic mechanism by the mechanical stimulation of the motor fibers by the ligature and not an escape of esophagus from tonic central inhibition.

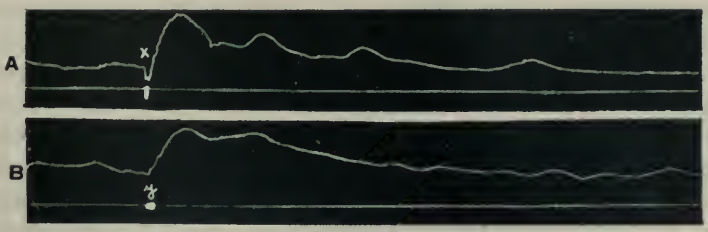

Fig. 15. Graphic record of the esophageal movements of a frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Animal fastened on back and abdominal and thoracic contents exposed. Hook *under* lower end of esophagus, attached to delicate recording lever. *A*, at *x*, ligation of one vagus. *B*, at *y*, electrical stimulation of the same vagus with a tetanizing current of moderate strength. Showing marked similarity on the esophageal movements between ligation and electrical stimulation of the same nerve.

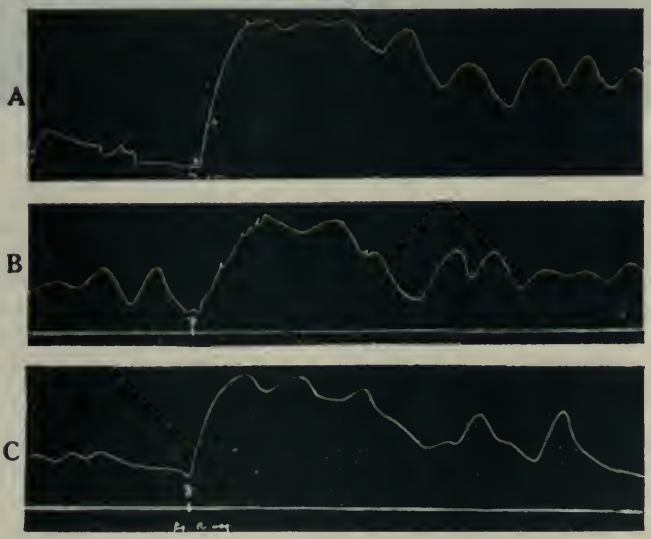

Fig. 16. Graphic record of the movement of the esophagus of a frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Stomach transected 0.5 cm. from cardia and attached at that point to delicate lever. *A*, at *x*, ligation of left vagus nerve in the neck. *B*, at *y*, careful introduction of a ligature under the right vagus 50 minutes after *A*. *C*, at *z*, ligation of right vagus 20 minutes after *B*. Showing in *B* the similarity in movements of the esophagus following mechanical stimulation of the nerve with ligation of the nerves (*A* and *C*) still connected with the medullary centers.

had a similar effect. The ligation of this nerve subsequently at z, in C, caused a motor response in the esophagus similar to the ligation of the first vagus nerve prior to its physiological division. It is furthermore plain that the hypertonus and motor activities are but of short duration.

The effect of sudden destruction of the medulla by chloroform. To eliminate as far as possible direct stimulation of the motor fibers of the vagus directly by ligation or indirectly by pithing of the medulla with a wire, we performed several experiments in which we destroyed the medullary centers suddenly and completely by injecting chloroform directly into them. Assuming that under these conditions the vagus centers would be rapidly destroyed without experiencing a temporary

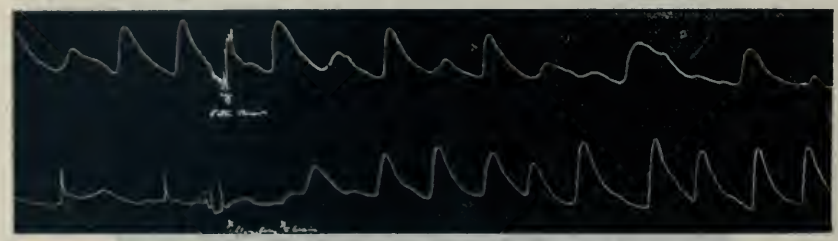

Fig. 17. Graphic record of the esophageal movement in the frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Upper jaw cut away just anterior to the eyes thus exposing anterior ends of cerebral cavity. Animal on back. Stomach attached near cardia to delicate lever writing on smoked surface. At x, intramedullary injection of one drop of chloroform through opening in anterior end of cranial cavity; at y, pithing medulla with a wire through the same orifice. Indicating an escape of the esophagus (and stomach) from the tonic inhibitory control of the vagus centers by destroying these centers suddenly with chloroform at x, for subsequent pithing of centers at y, had no further effect on the rhythm initiated by the chloroform destruction.

stimulation, all motor effects resulting from the destruction would indicate release of the esophagus and stomach from the normal tonic inhibitory control exerted by them.

Figure 17 gives the graphic results of such an experiment. Here (lower line) both the esophagus and stomach were relatively quiescent to begin with, the sharp upstroke prior to x representing movements of the head and neck of the animal. Immediately following the destruction of the medullary centers with chloroform at x, a pronounced rhythm appeared particularly in the esophagus which persisted throughout the period of experimentation. The subsequent mechanical maceration of the medulla with a pithing needle at y (upper line) had no further

effect. Experiments of this type indicate that the vagus centers exercise a tonic inhibitory control over the stomach and especially the lower end of the esophagus.

3. Changes in outline of the esophagus and stomach following ligation of the vagus nerves in the neck. The methods used for the graphic record of the stomach and esophagus following division of the vagi or destruction of the medullary centers involve tension on these structures. It seemed desirable, therefore, to show that the results recorded above transpired when the esophagus and stomach were resting in their normal positions within the abdominal cavity without the disturbing factors of diminished blood supply and traction on these structures. By a method previously described we found it possible to sketch rapidly the outlines of these viscera and thus obtain a consecutive record of the movements following ligation of the vagus nerve which would mean more to the reader than any amount of description.

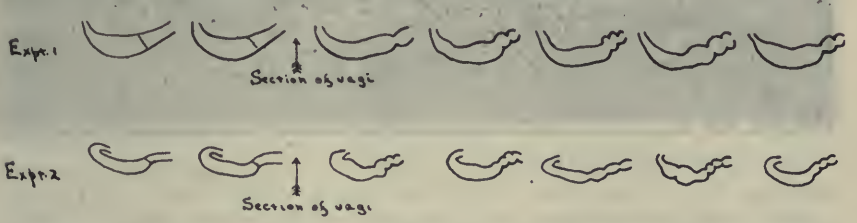

Fig. 18. Frog. Tracings of the outline of the esophagus and stomach before and after section of the vagus nerves. Showing hypermotility of the esophagus following isolation from the central nervous system.

Two experiments of this type follow:

Experiment 1. February 3 (fig. 18). Frog decerebrated and spinal cord pithed below the medulla. Animal fastened on its back. Thoracic and abdominal viscera exposed. Left lung excised. Ligatures under both vagi. Sketches of the esophagus and stomach made on a ground glass plate. Interval between sketches about 5 seconds.

Immediately following the ligation of the vagus nerve the contour of the quiescent esophagus changed. The esophagus shortened. Peristaltic waves swept over it for about 45 minutes. Occasionally the peristaltic waves passed over the stomach. The appearance of the esophagus and stomach immediately before and after ligation of the vagus nerves is shown in the upper series of sketches of figure 18.

In the second experiment the animal was prepared in a similar manner. In this animal, however, we excised the heart, lungs and liver. In

this experiment the esophagus did not shorten especially following ligation of the vagus nerves. On the other hand, peristalsis started promptly and continued throughout the period of observation (about 35 minutes). Sketches shown in lower line of figure 18 note the changes in the esophagus and stomach which followed ligation of the vagus nerves.

4. Incomplete tetanus of the esophagus and stomach on stimulation of the peripheral end of the vagus nerve with a tetanizing current. Figure 19 is offered as an example possibly of incomplete tetanus of the esophagus on electrical stimulation of the vagus nerve. The prolonged stimulation of the vagus at *y* leads to a prolonged hypertonic state of the esophagus on which were superimposed the individual contractions of automatic

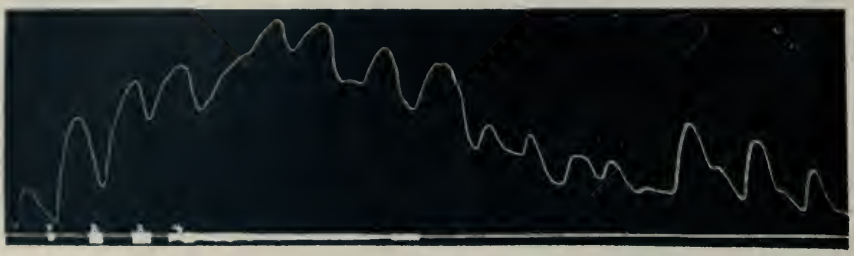

Fig. 19. Graphic record of the esophageal and, in part, gastric movements of the frog. Frog decerebrated. Spinal cord transected below medulla and pithed. Animal fastened on its back. Abdominal and thoracic viscera exposed. Stomach transected 0.5 cm. from the cardia and upper end attached to delicate lever writing on a smoked surface. Both vagi sectioned. *x*, stimulation of peripheral end of vagus with tetanizing current of moderate intensity. *y*, prolonged stimulation of the same vagus with the same strength of current. Showing incomplete tetanus of the esophagus following prolonged vagus stimulation.

rhythm. In view of the fact, however, that a very brief period of stimulation can produce a similar contracted state of the esophageal musculature lasting for some time after cessation of the stimulation, it is not so certain that the prolonged period of stimulation is responsible for the result which was obtained in this instance.

5. Effect of section and stimulation of the cervical sympathetic and splanchnic nerves on the motility of the esophagus and stomach. The results of section and stimulation of the cervical sympathetic had no pronounced effects on the movement of the esophagus and the stomach. A similar statement applies to our experience with stimulation of the peripheral end of the splanchnics. We attach no positive significance to our negative results.

6. The action of various drugs on the neuro-muscular mechanism in the esophagus of the frog: Nicotin. We have no extensive series of experiments relative to the action of this drug on the state of activity of the esophagus. Since nicotin abolished the central inhibitory control of vagus on the lung of the amphibia, we injected the drug intravenously in a few frogs to note whether the quiescent esophagus would escape as evidenced by marked shortening and the appearance of peristaltic waves. In 2 mgm. doses this effect was produced but it is obvious on the basis of our results that the increased motility might be due to direct stimulating action of the drug on the peripheral neuro-musculature apparatus. For where the evidence for the presence of inhibitory nerves in the vagi to the esophagus is wanting or doubtful, the description of the escape of the esophagus from their control by the use of nicotin is hardly justified.

Atropin. Since the stimulation of the peripheral end of the vagus invariably gave a marked motor response it seemed possible to paralyze the peripheral terminations of this nerve by suitable doses of atropin. Under such conditions we might be able to demonstrate the presence of inhibitory fibers.

Atropin failed to have the slightest recognizable effect on the peripheral motor terminations of the vagus. Even after giving the animal 10 mgm. of this drug intravenously, stimulation of the peripheral end of the vagus with a tetanizing current sent the esophagus into tetanus (fig. 20 at *x*).

Since the intravenous injection of the drug was made under conditions of possibly poor circulation through the esophagus, we injected three frogs subcutaneously with 8, 10 and 20 mgm. of the drug one hour before preparing them for graphic registration. In every case the results were the same. One frog which had received 20 mgm. at 9:15 was given another 20 mgm. of the drug subcutaneously at 4:30 p.m. At 4:50 p.m. when the respiration had ceased the animal was prepared for graphic registration. Even before section of the vagi or destruction of the medulla and spinal cord lively movements of the esophagus and stomach were in evidence. Section and stimulation of the vagi gave the usual motor effects. In fact, we were able to tetanize the esophagus by electrical stimulation of the nerve. However the results are interpreted, one thing seems clear, namely, that the motor termination of the vagus cannot be paralyzed by atropin.

Adrenalin. This drug even in dilutions of 1:100,000 exerts, when applied locally to the esophagus, a marked inhibitory effect on the periph-

eral rhythm present in the esophagus as is illustrated in figure 21 at x. Nor is the inhibitory action entirely due to the chloretone content present in the dilute solution of adrenalin used; for irrigation of the esophagus with a chloretone solution of equal concentration as at y of figure 21 produces only a slight and fleeting inhibition.

When the concentration of adrenalin is increased to 1: 10,000 and applied locally the inhibition is even more pronounced and long lasting. Even more remarkable is the fact that during the marked inhibition following irrigation, stimulation of the vagus nerve with any strength of current is without effect as is shown at y and z in A of figure 22. As

Fig. 20. Graphic record of the peripheral rhythm of the esophagus and stomach of a frog. Animal decerebrated. Spinal cord transected below the medulla and pithed. Both vagi have been sectioned and the peripheral end of one retained for stimulation. Twenty minutes before taking this record the animal received an intravenous injection of 10 mgm. of atropin sulphate through the anterior abdominal vein. At x, stimulation of the peripheral end of the vagus with a tetanizing current of moderate strength. Showing that atropin failed to paralyze the peripheral motor terminations of the vagus.

the action of the adrenalin wears off stimulation of the vagus nerve gives the usual motor effect. This is shown in B of figure 22, where the electrical stimulation of the vagus, x, for 3 seconds was promptly followed by motor effect. The irrigation of the esophagus at y with adrenalin led to an inhibition during which an electrical stimulation at x gave only a feeble motor response.

From this it would appear that adrenalin not only abolishes a peripheral rhythm of the esophagus however induced but, when administered in sufficient concentration, paralyzes temporarily the motor terminations of the vagus.

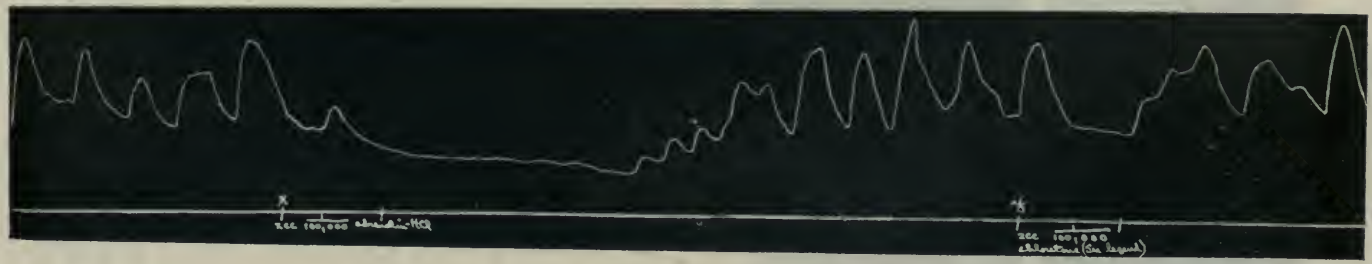

Fig. 21. Graphic record of the esophageal movements of a frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Vagi sectioned and medulla destroyed by pithing. Stomach transected 0.5 cm. from the cardia and attached at that point to delicate lever writing on smoked surface. *x*, irrigation of the esophagus with 2 cc. 1:100,000 adrenalin chloride solution during period indicated by the signal magnet. *y*, irrigation of esophagus with 2 cc. of chloretone solution equal in concentration to the concentration of chloretone present in 2 cc. of 1:100,000 adrenalin chloride solution. Showing that the inhibition by the adrenalin chloride solution is not due to its chloretone content.

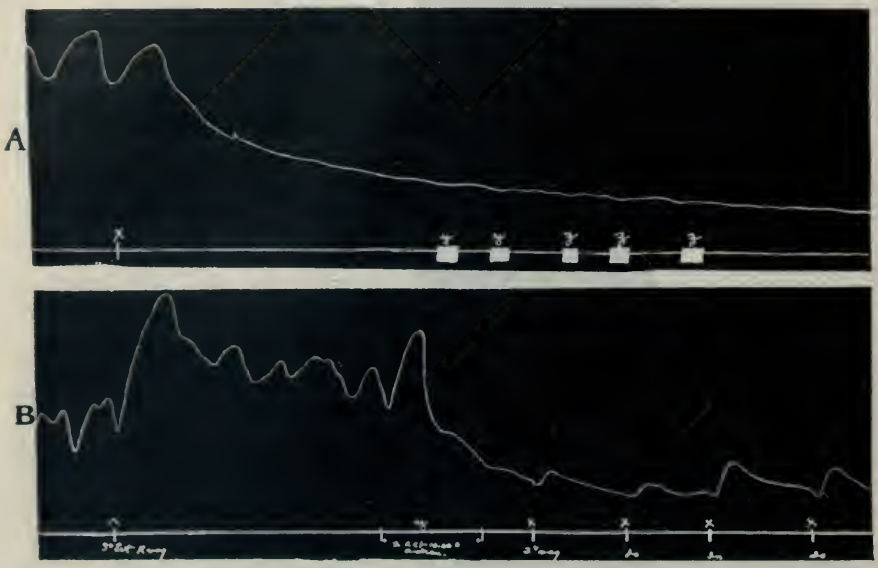

Fig. 22. Graphic record of the esophageal and gastric movements of a frog. Decerebrated. Spinal cord transected below medulla and pithed. Fastened on back and thoracic and abdominal viscera exposed. Both vagi sectioned. Stomach transected 0.5 cm. below cardia and upper end attached to delicate lever. *A*. Animal had received 10 mgm. atropin sulphate through cannula in anterior abdominal vein. This amount failed to paralyze the terminations of the vagus (see fig. 20). At *x*, irrigation of the stomach and esophagus with 2 cc. of 1:10,000 adrenalin chloride solution; at *y*, stimulation of the peripheral end of the vagus with weak tetanizing current; at *z* stimulation of the vagus with a strong tetanizing current. Showing adrenalin inhibition of the peripheral rhythm of the esophagus and stomach as well as paralysis of the motor termination of the vagus by this drug. *B*, at *x*, electrical stimulation of the right peripheral end of the vagus for 3 seconds with a tetanizing current of moderate intensity before and after the irrigation of the esophagus and stomach at *y* with 2 cc. 1:10,000 adrenalin chloride solution. Showing motor effects on electrical stimulation of the vagus, adrenalin inhibition of the esophageal and gastric rhythm and partial paralysis of the motor terminations of the vagus by adrenalin with beginning recovery.

DISCUSSION OF THE RESULTS

The results on the turtle are clear cut and convincing. There can be no doubt that the local motor automatism of the esophagus is held in tonic inhibitory check by the vagi, so that on section of the vagi, or destruction of the medulla, the circular musculature of the esophagus goes into hypertonus lasting throughout the experiment. In

line with this, stimulation of the peripheral vagus inhibits the circular musculature, including the cardia. This type of motor control of the reptilian esophagus and cardia is identical with the motor control of the amphibian lung.

It is true that extensive dissection is required in the turtle to permit satisfactory experiments of this type but we cannot see how the trauma can account for any essential part of our results. It seems more likely that the trauma to the nervous system depresses the medullary centers so that the hypertonus of the esophagus following section of the vagi is actually less than normal. The esophageal hypertonus is not due to local stimulation of the esophagus by the slightly inflated balloon, as the empty esophagus goes into hypertonus on vagi section.

The results on the frog cannot with certainty be interpreted as showing a predominating vagal inhibition of a local esophageal automatism. There is a remarkably prolonged motor effect on the esophagus of momentary stimulation of the vagus, a factor not recognized by Goltz and his followers. On the other hand, the marked inhibitory action of the drug adrenalin may be interpreted as demonstrating an inhibitory nervous mechanism. Our failure to demonstrate extrinsic inhibitory nerves in the vagi, cervical sympathetic or splanchnics, by section or by stimulation, may be due in part to the condition of the frog. The work was done during the winter months and the animals were not in the best of condition. But the temperature itself was not a factor, as some of the animals were slowly warmed up to room temperature before the experiment.

The reader may recall that in frogs in poor physiological condition the tetanus of the lungs, so marked in good preparations, may be insignificant or altogether absent. It seems reasonable to suppose that the inhibitory mechanism for the esophagus would be similarly depressed by poor condition of the animals, and hence to be demonstrated only in the very best of preparations. Peripheral "shock" may also be a greater factor in the esophagus than in the lung. It should be noted, however, that the frog with section of both vagi swallows air into the stomach, but cannot force the air into the contracted lungs (Patterson). This would seem to indicate that the hypertonus of the isolated lung is greater or of longer duration than the hypertonus of the esophagus isolated from the medulla.

Contrary to our results on the amphibian lung and the indications in the literature on mammals, neither atropin nor nicotin were of any service in differentiating between motor and inhibitory nervous mechan-

isms to the esophagus in frogs and turtles. These drugs do not paralyze the vagus motor fibers to the frog's esophagus or the vagus inhibitory fibers to the turtle's esophagus. Adrenalin paralyzes the gastric motor fibers of the vagi both in the turtle and the frog, but not the vagi inhibitory fibers to the turtle esophagus.

TABLE 1

Primary action of vagi section, of vagi stimulation, and of certain physiologically important drugs on the motor mechanism of stomach, esophagus, lungs and heart. Stimulation or increased action = +; inhibition = −. When both augmentation and inhibition may result the predominating action is placed above or first

	TURTLE				FROG				SALAMANDER	
	Stomach	Esophagus	Lung	Heart	Stomach	Esophagus	Lung	Heart	Lung	Heart
Section of vagi	+a −	+a −?	+?	+a	+a −	+a	+a	+a	+a	+a
Stimulation of vagi	+ −	− +?	+	− +*	+ −?	+ −?	− +*	− +*	−	−
Adrenalin	−	+	−	+	−	−	−	+	−	+ ·
Histamine	+	−	+	−	−	−	+	−	− +*	−
Nicotin	+	−	+	−	?	?	−	−	−	−
Atropin	− −	+	−	+	?	?	?	+	?	?
Pilocarpin	+	−	+	−	+	+	?	−	?	?

a, escape from inhibitory control of vagi. * after nicotin.

The striking lack of parallel between vagi and drug action on stomach, esophagus, lungs and heart may be recapitulated in tabular form (table 1).

The present results strengthen the view that *the primary action of many drugs on visceral motor mechanism depends on the predominant type of innervation (motor or inhibitory) of these organs.* The action of adrenalin on the turtle's esophagus appears, however, to run contrary to this view, as the predominating vagus influence is inhibitory, but adrenalin nevertheless augments the esophageal tonus.

On examining this table carefully it will be noted that histamine, nicotin and pilocarpin have an action on the stomach, esophagus, lung and heart of turtle directly opposite to the action of adrenalin and atropin. This direct antagonism of the drugs mentioned was particularly striking between adrenalin and pilocarpin.

The present results on the turtle's esophagus, together with the indications in the literature of at least temporary hypertonus of esophagus and cardia in some mammals following section of the vagi, show that the factors of local automatism and extrinsic inhibitory nerves must be taken into account in motor disturbances of esophagus and cardia. But experimental results on one species cannot, without further consideration, be transferred to another species, because of the evident variations in the degree of primitive motor control retained by this end of the gut (including the lung) in different animal groups.

SUMMARY

Turtles: 1. Section of the vagi or pithing of the medulla leads to hypertonus of the esophagus (circular musculature) and cardia. Stimulation of the vagi causes inhibition of the esophagus (circular musculature) and cardia. The predominant vagus innervation of the esophagus is therefore inhibitory, and this mechanism is in tonic activity.

2. The observations of Bercovitz and Rogers that section of the vagi may induce hypertonus and initiate contractions of the stomach, and that stimulation of the vagi causes inhibition of the stomach are confirmed, but the predominating action of the gastric vagi is motor.

3. Atropin and nicotin do not paralyze the vagi inhibitory fibers to the esophagus.

4. Adrenalin stimulates the esophagus, inhibits the stomach and paralyzes the gastric motor fibers of the vagi. The drug does not paralyze the vagi inhibitory fibers to the esophagus.

5. Nicotin, atropin, histamine and pilocarpin have opposite actions on the stomach and the esophagus.

6. No evidence of sympathetic innervation of the esophagus was obtained.

Frogs: 1. Section of the vagi causes in frogs a hypermotility of the esophagus particularly, but to some extent also of the stomach. Our evidence would seem to indicate that this hypermotility is due in part to an escape of these structures from the tonic inhibitory control of the medullary centers but essentially to the mechanical stimulation of the motor fibers carried by the vagi to these structures.

Stimulation of the peripheral end of the vagus nerves give invariably pronounced motor effects on the esophagus and stomach. An incomplete tetanus of the esophagus may result from such stimulation.

We could secure no direct evidence that the vagus and cervical sympathetic nerves carry inhibitory fibers to the esophagus and stomach of the frog. No positive significance is attached to these negative findings.

2. In the frog atropin even when used in large doses fails to paralyze the motor termination of the vagus nerves.

Adrenalin, on the other hand, promptly inhibits the peripheral automatic activity of the esophagus and stomach. This drug likewise temporarily paralyzes the motor termination of the vagus.

General. 1. The present data afford additional evidence that the primary action of many drugs on visceral motor mechanisms depends on the predominant innervation (motor or inhibitory) of the organs.

2. The present data indicate that tonic inhibitory innervation via the vagus nerves plays a rôle in the motor control of the esophagus and the cardia. But the conditions found in one animal group or species do not necessarily apply to another group or species, as the degree of differentiation in the motor control from the primitive condition appears to vary greatly in different species.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

V. The Nature of the Action of KNC on Paramecium and Planaria, with an Experimental Test of Criticism, and Certain Explanations Offered by Child and Others[1]

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Received for publication June 6, 1921

Successful experimentation in physiology generally depends to a very large extent upon the degree to which the process under investigation can be experimentally isolated. One of the means which can be used in this sense, to isolate the process of respiration in protoplasm, is the use of cyanides. It has become a well-known fact that cyanides in general change the rate of oxygen consumption and carbon dioxide production to various degrees depending upon conditions. The effect which is best known is the inhibitory action of potassium cyanide. Little if anything is known about its apparent tendency under certain conditions to accelerate the rate of respiration.

It has sometimes been tacitly assumed if not expressly stated, on the basis of good and extensive, but not complete experimental evidence that *all* biological oxidation processes are inhibited more or less by cyanide. There has been a tendency to assume that because KNC inhibits cell respiration in some or many organisms, therefore it should inhibit the respiration in all types of living protoplasm. See for example Hyman (1) Child and Hyman (2, p. 217).

It was therefore somewhat of a surprise to the writer when he was unable to demonstrate any inhibitory action of KNC on the oxygen consumption of Paramecium (3). These experiments suggested the advisability of measuring the effect of cyanide upon Planaria which had been used by Child and his co-workers in extensive studies in

[1] The writer is indebted to Mr. Emmett Rowles for valuable assistance in many of the experiments in this work.

which solutions of cyanide were used to determine supposed differences in rate of metabolism.

The experiments by Allen (16) showed that a perfectly reversible inhibition amounting to 70 or 80 per cent of the normal oxidation could be obtained. These results were confirmed by Hyman (1).

Since that time numerous papers have appeared in this and other journals by Child (4), (5), Robins and Child (6) and Hyman (7), (8), (9), in which various attempts are made at an explanation of the apparent discrepancy between the experimental results obtained from Paramecium and those from many other cells and organisms typically represented by Planaria. These criticisms and suggested explanations of the results may for convenience be classified under two heads.

First: Criticism of experimental method and interpretation of the data from the experiments on Paramecium. See Hyman (1), Child (5, footnote p. 155).

Second: Attempts by Child (4, p. 255 and footnote, p. 256) at explanations as to how these differences in experimental results between Paramecium and other organisms might come about. Repetitions of these criticisms and explanations have been made in several papers by Child and Hyman at different times; therefore I take it that in the minds of these writers the explanations which they offer constitute more than mere tentative suggestions. It will be the purpose in this paper briefly to answer the criticisms and show that the explanations which have up to the present been offered, can not possibly be correct. In a following paper results of experiments recently completed which give in the opinion of the writer the correct interpretation of the behavior of Paramecium toward cyanides will be presented.

Criticisms of the accuracy of experimental methods. In order that the reader may be able to see in the simplest way the writer's attitude toward the criticisms by Hyman (1, pp. 356–358) and Child (5, footnote p. 155), these specific criticisms are summarized and numbered, and the answer to each one is given briefly as follows.

1. It is stated that the solutions of KNC used by the writer in the tests on Paramecium were too dilute to affect the rate of oxygen consumption since Paramecium is more resistant to KNC than other organisms and dies only in relatively very high concentrations of cyanide. The answer is that a wide range of concentrations was used, as shown in the tables (3). The higher concentrations which were used *did* kill the animals although no trace of inhibition was evident. They were lethal concentrations, and that is all that is necessary in this connection

to prove the point that death in KNC solutions can take place without inhibition of the oxidations.

2. Child and Hyman state that it is probable that the alkalinity of the KNC solutions has a stimulating effect on respiration in Paramecium. This, however, is not correct as will be shown below.

3. It is stated that the oxygen consumption by protozoa is very small, therefore quantitative measurements are difficult to perform. Presumably Hyman means the *rate* of oxidation. The truth of the latter statement has never been shown, in fact the opposite may be true. If her statement means that the total quantities of oxygen consumed by the Paramecia during the experiments are too small to be accurately measured, then the answer is that the quantity consumed varied from about 15 to over 50 per cent of the total oxygen content of the water in the bottle. A glance at the tables will show this. The experimental error in Winkler's method when properly used is, as is well known, about 1 to 2 per cent.

4. The initial effect of KNC might be quite opposite to its final effect and therefore a test of the total oxygen consumption, such as that which was made by the writer in any one experiment, does not give any indication of what the time course of the effect of the cyanide really was. The answer is obviously that the experiments given were never designed to show what is the time course of the effect of cyanide on Paramecium. They do show that the toxic solutions of KNC had no more inhibitory effect than the non-toxic concentrations, which is the point in question. In fact, the toxic solutions had an accelerative effect, if any. This for example is true in the 10-hour period and also in the 29-hour period in table 3 of the experiment.

5. The error due to iodine absorption by the cells was not adequately determined, so that it could not be adequately applied as a correction to the results. The answer is that for the essential purpose of the experiment and conclusions drawn the iodine error may be ignored entirely, for we are primarily comparing the oxygen consumption in low and high concentrations of cyanide. See for example table 3, column 7, in which the iodine error is necessarily the same in all the determinations.

6. To Hyman a very important lack in the experiments is that "no figures are given anywhere of the normal rate of oxygen consumption of the same lots of Paramecia" (p. 357, l. c.). Proof that different 1 cc. samples of the same Paramecium suspension consume identical amounts of oxygen under the same conditions will be found in, for exam-

ple, table 4, column C. Several other experiments in an earlier paper should make this clear to any one (cf. Lund (10)). It is evident from this that in the experiments referred to by Hyman it was not at all necessary to test the oxygen consumption by the same lots of Paramecia.

7. In my paper (3) it was suggested as probable that after cytolysis of Paramecium, those oxidations ceased which especially represented that part of the respiratory exchange of a cell which is directly concerned with transformation of chemical energy into, for example, mechanical work.

TABLE 1

Showing the difference in total output of CO_2 by normal Paramecia and by the same number of Paramecia after mechanical disintegration. The latter was a colloidal solution, no cell fragments present. Duration of tests 2½ and 3 hours. Total CO_2 liberated is given in equivalents of cc. n/100 HCl

EXPERIMENT	BOTTLE	CONTROLS		5 CC. DISINTEGRATED PARAMECIA IN TAP WATER
		Blank; 5 cc. tap water; no Paramecia	5 cc. normal Paramecia in tap water	
		cc. n/100 HCl	*cc. n/100 HCl*	*cc. n/100 HCl*
I	1	0.20	0.85	0.21
	2	0.17	0.98	0.22
	3		0.98	0.13
	4			0.20
Average.....		0.18	0.93	0.19
II	1	0.20	1.00	0.29
	2	0.20	1.13	0.35
	3		0.84	0.33
	4			0.29
Average.....		0.20	0.99	0.31

The suggestion was based upon the observations of Warburg (11) on the respiration of mechanically disintegrated fertilized eggs, and of Fletcher and Hopkins (18) on disintegrated muscle. This suggestion, it is maintained by Hyman, is unwarranted. Accordingly, in order to obtain further direct evidence on this point from Paramecium, a few experiments were made in which the rate of CO_2 elimination by identical lots of Paramecia were compared. One of the lots was mechanically disintegrated and the other left normal as a control. The animals were mechanically disintegrated in tap water by means of a small circular knife running at 3000 revolutions per minute. No trace of cell

fragments remained, the suspension was made up of small granules and was colloidal in character. The method for determination of CO_2 was a modification of that described in a former paper (12). Four tests were made, all of which gave the same results; two of these are given in table 1.

It is clear from the results that under the conditions of the experiment the CO_2 elimination in Paramecium practically disappears after destroying the protoplasmic structure of the cell. This fact along with the data given in table 4 (3) strongly supports the suggestion which was originally made, that stopping of the greater part of normal cell respiration follows cytolysis even in the absence of KNC. Similar evidence was obtained when high oxygen concentration was used as a toxic agent (10).

The problem of the relation of protoplasmic structure to cell respiration is, however, in need of extensive and accurate investigation before it will be possible to lay down any general conclusions.

Is the toxic action of KNC solutions on Paramecium due to their high alkalinity? The only criticism made by Hyman and Child, that would seem to have any justification, is that given under criticism no. 2 above, where it was maintained the alkalinity of the cyanide solutions might affect cytolysis and also the rate of respiration in Paramecium.

It would appear possible, since no inhibitory effect on the respiration could be detected in concentrations of KNC from m/27400 up to as high as m/274, and since the animals began to die within 30 hours in m/274, that the high alkalinity was the cause of death rather than the action of cyanide as such. An answer to this question can readily be given by comparing the survival time of identical lots of Paramecia from the same suspension in equimolecular solutions of KNC and KOH, whose hydrogen ion concentrations are known. The following table gives the result of one experiment from among several which were carried out with identical results.

It is clear from the table that the high toxicity of the KNC solutions is not due to their high alkalinity, for the KOH solutions whose pH was even slightly higher than that of the KNC solutions did not kill any of the animals within the duration of the experiment. We must conclude therefore that the toxicity of KNC solutions used in the experiments on the rate of oxidation in Paramecium was not due to the high alkalinity but that the toxic action of the higher concentrations of KNC in which no trace of inhibition of respiration was found, was due to the toxicity of the cyanide as such.

Does the hydrogen ion concentration of the medium have any effect upon the rate of oxygen consumption by Paramecium? It has been suggested by Child (5, footnote, p. 155, and in private communication) that cyanide solutions, due to their high alkalinity, might stimulate the cell to a higher rate of oxidation which therefore might counteract or conceal

TABLE 2

Showing the relative survival times of Paramecium caudatum in the same concentrations of KNC and KOH. The table also shows the absence of a relation between survival times and pH of the solutions. Volume of each solution 100 cc. pH of tap water = 8.2. x = few dead. xx = many dead. xxx = most dead. 0 = all dead

Cubic centimeters of m/10 KNC to 100 cc. tap H_2O	pH	Time in hours												
		0	3	7	23	25	29	37	59	71	83	107	134	153
0.2	8.2							x	xx	xx	xx	xxx	xxx	0
0.4	8.4							x	xx	xx	xxx	xxx	xxx	0
0.6	8.8							x	xx	xxx	xxx	xxx	xxx	0
0.8	9.0						x	xx	xxx	xxx	xxx	0	0	0
1.0	9.2					x	xx	xxx	0	0	0	0	0	0
2.0	9.4				x	xx	xxx	0	0	0	0	0	0	0
Cubic centimeters of m/10 KOH to 100 cc. tap H_2O														
0.2	8.4													
0.4	8.6													
0.6	8.9										All living and normal			
0.8	9.2													
1.0	9.4													
2.0	9.6													

the characteristic inhibitory effect of the cyanide. While this might appear at first sight a plausible explanation of the apparent absence of inhibition of the rate of oxidation in Paramecium, after a little reflection upon the striking uniformity of the numerical values, given for example, in column 7 of table 3 (3), it becomes evident that it would be

a strange coincidence if in concentrations of cyanide ranging from m/27400 to m/274, the inhibitory effect of the cyanide and accelerative effects should *just balance one another* in practically every concentration between and including these limits! However, not being content with leaving this question without experimental test, I give below the results

TABLE 3

Showing the rate of oxygen consumption by Paramecium caudatum and its independence of the hydrogenion concentration in the medium. Paramecia used in this experiment were transferred to tap water and starved 18 hours previous to the experiment. Volume of bottles 136 cc. Temperature 19°C. Duration of test 36 hours. Range of pH of tap water to highest concentration of KOH in tap water was 8.2 to 9.5. The numbers in the table represent cubic centimeters of thiosulphate equivalent of oxygen

BOTTLE	CONTROLS			ANALYZED AT END OF 36 HOURS; 2 CC. PARAMECIA ADDED TO EACH BOTTLE						REMARKS
	Analyzed at once		Analyzed at end of 36 hours. 2 cc. paramecia added. No KOH	Cubic centimeters of m/10 KOH added						
	Blanks	2 cc. Paramecia added		0.2	0.4	0.6	0.8	1.0	2.0	
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	4.60	3.38	2.75	2.58	2.70	2.90	2.60	2.70	2.52	All Paramecia living and normal at end of test
2	4.50	4.30	2.65	2.80	2.60	2.70	3.00	2.70	2.90	
3	4.52	4.40	2.77	2.78	2.80	2.70	2.50	2.70	2.70	
Average...	4.54	4.36	2.72	2.72	2.70	2.76	2.70	2.70	2.71	
Average cubic centimeters of thiosulphate equivalent of O_2 consumed.			*1.64*	*1.64*	*1.66*	*1.60*	*1.66*	*1.66*	*1.65*	

of one experiment designed to determine whether the hydrogen ion concentration of solutions represented by those of the cyanide and alkali solutions employed in table 2 above, and similar to those in the experiments on oxygen consumption (3), does have any effect upon the rate of respiration.

The striking uniformity of rate of oxygen consumption by Paramecium in solutions of KOH whose range of pH is from 8.2 to 9.5, is evident from the figures representing the average oxygen consumption by identical 2 cc. lots of Paramecium suspension. There is no trace of a stimulating or accelerative effect on the respiration.

TABLE 4

Showing that the rate of oxygen consumption in solutions of KNC is not changed by the addition of an equivalent amount of HCl to reduce the alkalinity. A dense washed suspension of animals was used. Temperature 19°C. Duration of test 17½ hours. Numbers in table represent cubic centimeters of thiosulphate equivalent of oxygen

BOTTLE	CONTROLS				ANALYZED AT END OF 17.5 HOURS 2 CC. PARAMECIA ADDED TO EACH BOTTLE					
	Analyzed at once			Analyzed at end of 17.5 hours. 2 cc. Paramecium added	Cubic centimeters m/10 KNC added			Cubic centimeters of m/10 KNC+ cubic centimeters of m/10 HCl added		
	Blanks	2 cc. Paramecium added	2 cc. Paramecium + 2 cc. m/10KNC + 2 cc. m/10HCl		0.4	1.0	2.0	0.4 + 0.4	1.0 + 1.0	2.0 + 2.0
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	4.70	4.42	4.55	1.55	1.0	0.75	1.02†	1.10	0.83*	0.96†
2	4.68	4.25	4.35	1.60	1.0	0.95	.70†	0.35	0.55*	0.80†
3	4.75	4.40	4.45	1.35	0.6	0.55	1.20†	0.40	0.65*	1.25†
Average...	4.71	4.36	4.41	1.50	0.86	0.75	0.97	0.61	0.67	1.00
Average O_2 consumed cubic centimeter of thiosulphate				2.86	3.50	3.61	3.39	3.75	3.69	3.36
Average of all					3.50			3.60		

† = Less than 2 per cent of Paramecia dead.
* = Very few dead.

Will neutralization with an acid of KNC solutions unmask the supposed inhibitory effect of cyanide upon the rate of respiration in Paramecium? A different way to isolate the supposed accelerative effect of high alkalinity from the supposed inhibitory action on Paramecium due to cyanide as such, is to determine whether or not solutions of different concentrations of KNC which have been brought to practically the same hydrogen ion concentration by adding a proper amount of acid (HCl), have any effect on the rate of respiration.

Three concentrations of KNC were used; these were made up by adding 0.4 cc., 1.0 cc. and 2.0 cc. m/10 KNC respectively to each bottle in three sets of three bottles each. Then 2 cc. of Paramecium suspension were added to each bottle of 137 cc. volume. A second set of bottles was prepared in the same way except that in addition to the KNC, an equivalent quantity of m/10 HCl was added to reduce the alkalinity of the KNC solutions. A set of controls without cyanide or acid was also supplied in order to compare the rates of oxygen consumption in cyanide, and neutralized cyanide, with that of duplicate samples of Paramecia in tap water. The results are given in table 4. The total consumption in $17\frac{1}{2}$ hours in KNC and neutralized KNC was about $\frac{7}{9}$ of the total quantity of oxygen in the bottle. There can therefore be no question whatever of the significance of the numerical values representing the average total oxygen consumption which is given in cubic centimeters of thiosulphate in table 4. The average oxygen consumption by the animals in tap water was equivalent to 2.86 cc. thiosulphate, the average of *all* the lots in KNC was 3.50 cc. and the average of *all* the lots in neutralized KNC was 3.60 cc. thiosulphate. No difference in the different concentrations of KNC is apparent. The solutions containing 2.0 cc. KNC and 2.0 cc. neutralized KNC were both about equally toxic as shown by the fact that some of the animals in both were dead at the time when the experiment was stopped. The death of animals and consequent decrease in the oxygen consumed is barely noticeable in the lower values of the average total oxygen consumed by the animals in the bottles to which 2.0 cc. of the KNC had been added.

The hydrogen ion concentrations in all the bottles which contained KNC and acid was very nearly the same as that of the tap water, as was shown by colorimetric tests. While the range of hydrogen ion concentration in the bottles containing 0.4 cc. 2.0 cc. KNC was 8.4 to 9.4. In this experiment there occurs an apparently distinct accelerative effect by the KNC on the rate of respiration. This acceleration amounts to about 25 per cent of the normal rate of respiration, but this accelerative effect is not due to the alkalinity of the solutions, as Child suggests. Further study of this phenomenon is necessary before any definite statements can be made as to the conditions for its occurrence and actual magnitude.

It will be clear from what has been presented thus far that the results from different experiments confirm one another, and in every detail confirm the results and conclusions of the writer's previous studies on the effect

of cyanide upon Paramecium. Furthermore all the suggested explanations by Child and Hyman of the difference in action of cyandie upon Paramecium and Planaria fail entirely to stand the test of experiment. The question still remains, why does KNC act differently upon Paramecium than upon Planaria? Some definite light will be thrown upon this question in a later paper.

A test of Child's explanation of the differences in survival time in KNC of fed and starved Paramecia and Planaria. It is a striking fact that fed Planaria, Didinia and Paramecia—and perhaps the same will be found for other animals—are more resistant to the toxic action of cyanide than starved animals of the same species. Similarly due to the action of food, the same animals after feeding have a higher rate of respiratory exchange than starved animals. The apparent discrepancy between these facts and Child's explanation of the relation of susceptibility to toxic agents and the rate of metabolism, or more specifically the rate of respiration, has been discussed in several papers by Child and his co-workers. It is maintained that in reality high susceptibility is correlated to high rate of respiration and low susceptibility to a low rate of oxidation. A full discussion of the question will be found in Allen (17). Child (4, p. 255 and footnote, p. 256) and Hyman (7, p. 398) have offered an explanation of the apparent discrepancy between theory and facts by making what appear to be two assumptions. First: That the toxic action of cyanide is primarily, if not entirely, upon the superficial structures of the animal, for example, in Planaria its action is assumed to be upon the body wall and in Paramecium the assumption is that the cyanide acts upon the ectoplasm. Second: It is assumed that when Paramecium or Planaria are fed, the increment of increase of respiration is localized largely if not entirely in the intestine of Planaria and the endoplasm of Paramecium, consequently the rates of respiration of the body wall and intestine in Planaria are more or less independent variables, a similar reasoning applies to endo- and ectoplasm in Paramecium. It is clear that since in the direct measurement of the respiratory exchange the results represent the algebraic sum of the rates of respiration in different parts of the cell or organism, it might conceivably be true that susceptibility tests with cyanide which according to the assumption acts only upon the body wall, might give different results from those obtained by direct measurement of the total oxygen consumption and yet not be out of harmony with Child's conception of susceptibility insofar as it may be related to rate of respiration.

This explanation may appear at first sight to have some merits, since it is probably certain that oxidations proceed at different rates in different parts of the same cell and since it is well known that different organs and tissues have different rates of respiration (19). However, there are two sets of facts which seem to speak fatally against this explanation of the difficulty.

First: Specific dynamic action of foods in higher organisms has been shown to be an accelerative effect upon the respiration of the body cells and not an effect residing in the alimentary tract, so that if we are permitted to reason by analogy from higher animals, we have certainly no *a priori* reason for believing that the effect of food on respiratory exchange in Planaria or Paramecium is localized in the intestine or endoplasm respectively.

Second: The most fatal objection is the fact that cyanide inhibits the oxidations in about equal percentage amounts of the normal rate, in both *starved and fed* Planaria. Now if, as we are told by Child and Hyman, the cyanide only or primarily acts upon the body wall and since food accelerates only or primarily the respiration in the intestine, then surely we should be able to note a very large and *distinct* difference in the degrees to which total respiration is inhibited in starved and fed Planaria when subjected to the same concentration of KNC, since according to Child and Hyman the action of cyanide is primarily on the body wall.

Tables 5 and 6 are two separate but similar experiments, the results from which show that the percentage inhibition in starved animals with 1 cc. m/10 KNC is 48 per cent and 47 per cent respectively, while the percentage inhibition by the same concentration of KNC in the corresponding fed lots of Planaria is 53 per cent and 63 per cent respectively. Evidently the KNC acts upon the respiration of internal organs as well as that of the body wall or else we must conclude that the specific dynamic action of food is on the body wall as well as internal organs. In short cyanide acts to a large extent, if not entirely upon the respiratory processes in the *same cells* whose respiratory processes are affected by the specific dynamic action of the food. The conclusion must therefore be that the interpretation of the difference in survival time in KNC solutions of fed and starved Planaria (and therefore also for Paramecium so far as Child's argument is concerned) on the basis of difference in rate of respiration in body wall and intestine is completely unwarranted. In fact, the percentage inhibition appears to be greater in fed than in starved animals which is directly opposite to what one would expect if the proposed explanation were correct.

TABLE 5

Comparison of the amounts of inhibition by KNC of the rate of oxygen consumption in fed and starved Planaria agilis. Animals starved 12 days before feeding beef liver to set B. Both starved and fed sets of animals were again starved 17 hours before the test. Duration of test 8½ hours. Volume of bottles 102 cc. Twenty animals in each bottle. The individual lots of 20 animals each were weighed just before feeding animals of set B. Numbers in the table are the actual number of cubic centimeters thiosulphate equivalent of oxygen consumed during the test. The weights of the different lots of animals were very closely the same and hence weights are not given in the table

BOTTLE	BLANKS. CONTROL = TOTAL O_2 AT BEGINNING	STARVED SET A		FED SET B		
		No KNC	1 cc. n/10 KNC added	No KNC	1 cc. n/10 KNC added	
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	3.25	1.63	0.90	2.36	1.17	All animals living and normal at end of experiment
2	3.25	1.68	0.90	2.26	1.08	
3	3.25	1.72		2.26	1.00	
Average consumed during test		1.68	0.90	2.29	1.08	
Average consumed per gram during ing test		6.64	3.46	9.27	4.31	
Per cent inhibition by KNC			*48*		*53*	

TABLE 6

Comparison of the amounts of inhibition by KNC of the rate of oxygen consumption in fed and starved Planaria agilis. Animals starved 14 days before feeding beef liver to set B. Both starved and fed sets of animals were again starved 15 hours before the test. Duration of test 8 hours. Otherwise the procedure was exactly as given in table 5

BOTTLE	BLANKS. CONTROL = TOTAL O_2 AT BEGINNING	STARVED SET A		FED SET B	
		No KNC	1 cc. n/10 KNC added	No KNC	1 cc. n/10 KNC added
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	3.20	1.52	0.77	2.16	0.84
2	3.15	1.44	0.88	2.24	0.84
3	3.20	1.48	0.84	2.16	
Average consumed during test		1.48	0.83	2.19	0.84
Average consumed per gram		7.87	4.12	11.34	4.20
Per cent inhibition by KNC			*47*		*63*

Further careful study and comparison of the effects of KNC on fed and starved animals may in all probability throw very important light upon the nature of the specific dynamic action of food.

CONCLUSION

It will be clear from the foregoing experiments which were designed to test briefly the validity of the criticisms and explanations offered by Child and Hyman, that not one of these criticisms or explanations has stood the test of experiment and careful examination. As a matter of fact a careful study of the data in the tables of the writer's previous papers should have obviated most if not all of the criticism in regard to the earlier experimental results. The writer has with some reluctance ventured into this reply to criticism since he has felt that in any situation where individual judgments differ in regard to the interpretation of experimental results, it is desirable to pay more attention to rigorous formulation of experiment than lengthy verbal discussion. From this standpoint the writer may be pardoned for this reply to criticism since he has attempted to answer by experiment rather than by explanations which are based on assumptions.

SUMMARY

1. Potassium cyanide even in concentrations which cause cytolysis do not decrease the rate of respiration in Paramecium caudatum. This confirms the writer's previous results. Paramecium therefore differs markedly from Planaria and most other cells and organisms whose respiration rate is decreased in the presence of KNC.

2. The criticisms offered by Child and Hyman of the experimental results and conclusions by the writer are shown to have no basis in fact.

3. The toxic action of KNC upon Paramecium is not due to the alkalinity of the cyanide solutions, but is due to the action of the cyanide as such.

4. The alkalinity of the solutions used in the experiments on Paramecium does not effect the rate of respiration, contrary to the suggestions offered by Child. Furthermore neutralized solutions of KNC are not different in their action from non-neutralized solutions of KNC. Whether or not KNC as such has a tendency to accelerate the oxygen consumption by Paramecium is an open question.

6. The assumption by Child that the rate of respiration in the body wall of Planaria is not primarily affected by feeding, and that KNC only or primarily affects the body wall and superficial structures, is not correct. For experiment shows that the percentage inhibition of the respiration in fed animals is just as great or even greater than the percentage inhibition in starved animals used as a control. In view of this fact similar assumptions which are made by Child and applied to endo and ectoplasm of Paramecium are unwarranted.

7. The explanation as to why Paramecium and Planaria differ in their behavior toward cyanide is to be sought in an entirely different direction from that suggested by Child and his co-workers. A following paper on the relation of oxygen concentration and its relation to the action of KNC will throw light on this question.

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GRADIENTS OF VITAL STAINING AND SUSCEPTIBILITY IN PLANARIA AND OTHER FORMS

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Received for publication June 3, 1921

The investigations described in this series were undertaken in an attempt to reveal more facts as to the nature of the metabolic factors controlling individual organization and development, and to assign to these factors their proper relative values. Since recent work along this line rests largely upon a definite background of previous work, particularly that of Child, some of the conclusions of this writer may be summarily reviewed, with the caution that no statement or interpretation should be attributed to him without first consulting his own writings (1), (2), (3).

After extended experimentation and careful analysis of phenomena of regulation, growth and development in many organisms, Child put forth certain helpful generalizations as to the dynamic nature of the organism, which were applied convincingly to most varied and apparently independent groups of data. According to this view it is held that: metabolism is the basis of the phenomena of life, and an axiate "organic individual in its simplest terms" consists of a quantitative "metabolic gradient, or gradients in certain metabolic reactions, perhaps oxidations, with associated protoplasmic conditions," existing along the main axis and probably also in minor axes; the establishment of such a physiological gradient or gradients by interaction of environment and specific protoplasm is the first prerequisite to development and organization, and constitutes the basis of the functional and structural symmetry and polarity of the individual; through transmission of excitations the region of highest metabolic activity in the axial gradient exerts a dominating and integrating influence over subordinate levels with a lesser metabolic rate, such dominance or control being manifested by a correlating, coördinating, and generally unifying action in ontogeny, growth, regulation, behavior, etc.

Proofs of the existence of such a metabolic gradient and evidence of its nature may be found in the literature cited. These proofs and evidences have hitherto concerned themselves chiefly with differences along the axis in regulation capacity, in susceptibility, in output of CO_2, in consumption of O_2, and in electrical potential. Numerous other differences, often closely associated with metabolic activity, might well be sought for and studied in favorable forms, e.g., differences in heat production, in electrical conductivity, in H ion concentration, in water content, in permeability of membranes, in state of dispersal of colloids, etc. The rôle of each of these factors deserves individual attention, especially because of the wide applicability of the results in physiological gradients and in metabolism generally.

The writer believed that an attack of the problem might be made by a study of the action of electrolytes and dyes. Certain aspects of H ion action have been treated (4), and results with salts will be reported later. The object of the present paper is chiefly to state the experimental facts as observed regarding gradients of staining and susceptibility in several flatworms, protozoa, hydra, annelids, and the chick embryo with vital and other dyes; to analyze and interpret as far as possible these results in their bearing upon the concept of metabolic gradients as a further test of its validity and applicability; and, specifically, *to ascertain whether regions of high general susceptibility and rapid respiratory exchange behave in a characteristic manner in the staining process* as shown by the diffusion, segregation, flocculation, etc., of the dyes. The results are believed to contribute additional proof, with agents hitherto little employed, of the reality of the metabolic gradient in the forms used, and further evidence as to the nature of these gradients, particularly with regard to certain physico-chemical properties associated with high metabolic activity.

The experiments were performed for the most part at the University of Chicago in 1916-1917; the paper was then put in substantially its present form. Now newer observations and more recent literature are included. While the writer naturally assumes full responsibility for the results embodied in this work, he gratefully acknowledges his debt throughout to Dr. C. M. Child for many kindnesses and for the unfailing suggestiveness of his writings and criticisms.

GENERAL STATEMENT OF RESULTS

As might perhaps have been anticipated, there is a rather clean-cut difference between basic and acid dyes in their staining capacities

intra vitam, corresponding to the known differences in their physical and chemical properties. Basic dyes alone truly and definitely stain the tissues of the organisms under observation; though acid dyes sometimes penetrate and are even stored in granules, they do not in general become visible by fixed staining of protoplasm. Basic dyes of most varied chemical constitution and relationship were used but, while they differed very considerably in toxicity, in irritating action and in details of staining, the final staining pictures obtained with all were essentially similar. Most basic dyes, as toluidin blue, Victoria blue, crystal violet, methylene blue, janus green, etc., and even neutral red, are much more toxic than the acid dyes, as congo red, eosin, erythrosin, trypan blue, methyl orange, acid fuchsin, orange G, etc.

A given tissue or layer does not stain uniformly and simultaneously throughout the length of the specimen, even though at final saturation just before death the intensity of stain may become approximately equal everywhere. Regions of strongly marked susceptibility to such lethal agents as had been used and to acids, alkalies, and the dyes themselves, are regions where the basic dyes first became detectable in certain granules or globules of the cells. Thus in general a staining gradient is produced indicating directly the metabolic gradient. A gradation of penetration was found with every major gradation of susceptibility.

Depth of coloration increases rapidly as the death point is neared, but preliminary to the actual onset of disintegration there occurs in most species with most basic dyes a sudden loss of both natural pigment materials and stained particles, leaving the most susceptible parts strikingly decolorized.

In causing a selective disintegration methylene blue and some other dyes proved to be favorable agents for demonstrating not only the chief longitudinal axis in flatworms but also in many cases the minor axes as well. But these axes were not always to be distinguished by differences in staining, nor is there noticable difference in rate of staining of young and old individuals.

EXPERIMENTAL

Methods. Dye samples were obtained from as varied sources as possible (Grubler's, also Bausch and Lomb's and Kahlbaum's). Stock solutions were made up in well or tap—rarely in distilled—water, and were not made free from the salt impurities with which they were dispensed.

In vital staining it is highly important that the experimental animals be healthy and that they be kept under almost continuous observation to

watch the progress of the staining since very erroneous notions may be obtained by examining the specimen at the end of the process when tissues have been loaded to saturation. The staining should be witnessed as a process rather than observed in the finished state. Animals were usually brought under observation in clear water and closely examined from time to time with eye, lens, dissecting or compound microscope, and were often flattened under a cover slip with or without support, or teased.

The salient features of *the susceptibility method* have been many times described. According to the concentration of chemical agents or the intensities of physical conditions used there are two general modes of studying relative susceptibility, the direct and the indirect, both of which seem to have a definite and characteristic relation to the metabolic rate. With the *direct* method such concentrations or intensities are used as are lethal within a few hours; in this case individuals or parts with highest metabolic rate are most susceptible, and the susceptibility gradient follows the metabolic gradient. With the *indirect* method such lower concentrations or intensities are used that some acclimation occurs and death takes place only after many hours or days; in this case acclimation is most rapid and complete in individuals or parts with highest metabolic rate and length of life varies directly with rate of reaction.

As *criteria of* relative *susceptibility* use was made of loss of motility or response to stimulation, possibility of recovery, etc. For most lower invertebrates disintegration is a satisfactory index, and appropriate for fairly exact and quantitative readings. Death is manifested by a loss of continuity of surface contour, swelling, and loss of constituents, by the visible separation of tissue fragments and cells from the main mass, and by change to acid phase of some dye indicators (e.g., neutral red). With the usual slight disturbances in the container tissue fragments continue to scatter out into the medium until little or nothing remains *in situ* of the dead parts. A disintegrating organism thus imparts a turbidity and a tinge of color to clear water. Impending disintegration is often indicated by cloudy swelling, opacity, immobility and loss of local responses. Accompanying color changes consist of loss of more or less natural pigment or previously stored stain. Criteria of equivalent staining rates are those of direct observation and readings on the time required for first visible coloration of a given part.

Factors modifying the effect of dyes: Concentration. Figure 1, summarizing averaged data collected from many protocols, shows the

fairly direct relation existing between concentration and time of first staining, staining at all levels, and first disintegration of *Planaria dorotocephala;* vital staining is roughly a function of time and concentration. The interval elapsing between a given intensity of staining and the initial disintegration widens with increasing dilution, until the

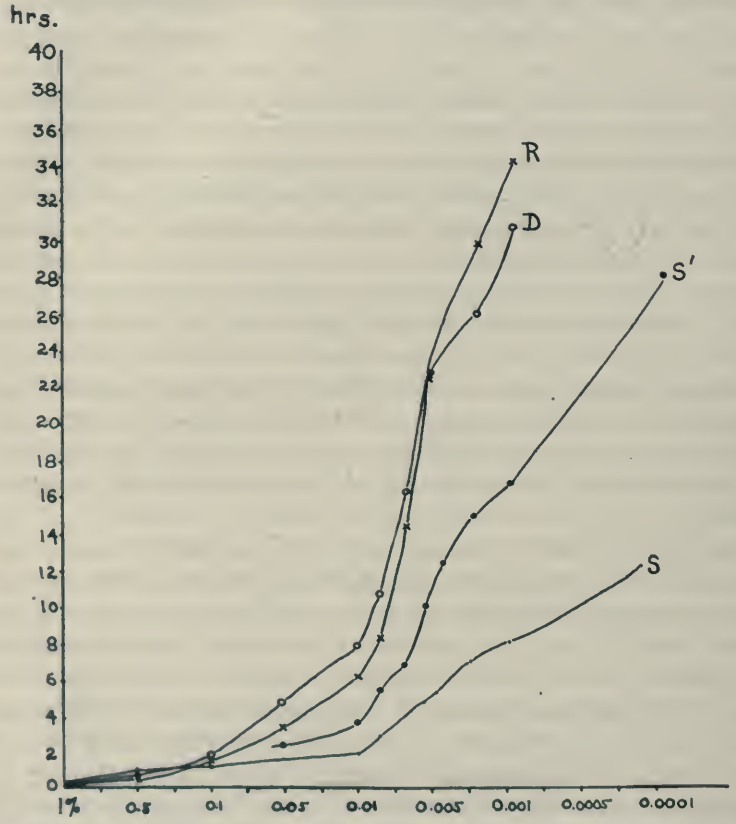

Fig. 1. Times of first visible staining, *S*, of first visible staining at all levels, *S'*, of first disintegration, *D*, and of longest possible exposure with complete or partial recovery, *R*, of *Planaria dorotocephala* of about 15–18 mm. length in different per cent concentrations of methylene blue at room temperature.

animals, though ultimately well stained, disintegrate less and less completely with greater individual variations, and finally near 0.0001 per cent will live on stained for an indefinite period either wholly intact or after recovery with loss of head and other most susceptible parts. In less toxic dyes like neutral red or even dilute methylene blue the worms

survive for months, carrying the dye even past fission and regeneration crises. In 0.1 per cent methylene blue loss of head substance occurs before stain has become at all visible in caudal regions, and in slightly higher concentrations toxic effects may be produced without appreciable visible staining, for recovery is impossible when staining begins. In short the curves of staining, S, S', do not run parallel with that of recovery, R, or that of initial disintegration, D. With a given intensity of stain prognosis for recovery is least favorable from strong and practically certain from weak solutions. Disintegration and failure to recover apparently depend less upon actual staining than upon presence of excess stain in the medium.

So far in this work the reversed order of susceptibility (indirect) has not been met with even in most dilute solution. But mere traces of methylene blue are said to have an accelerating effect upon growth of yeast.

Age of the organism is doubtless a factor in determining dye action. Ten large and ten small planarians were immersed together in 0.1 per cent methylene blue and examined at half-hour intervals and records made of the progress of their disintegration. A graph of the results shows a little difference in the resistance of the two sets of animals, the younger ones being perhaps slightly more susceptible than the larger. Similar results are obtained with acids (4).

Temperature. At 14°C. staining in 0.02 per cent m.b. is strikingly delayed as compared with that under the same conditions at 23°C. The protective effect of cold on organisms in the dye is so marked and the rates of staining and disintegration are so similarly modified that the clue might be followed further for evidence on the exact value of the temperature coefficients for intake of dye and for disintegration—particularly since amount of adsorption has a negative temperature coefficient (5). It would seem that adsorption is soon followed by chemical combination.

Hydrogen ion concentration. The reaction of a dye solution is of prime importance. Staining in well or lake water of pH = 7.5 may be considered rapid; if this water be made more alkaline by the addition of NaOH rate and depth of staining increases with rise of pH. At the same time in the more alkaline media differences in sites of staining also appear in that certain irregular or stellate bodies with nucleus-like centers stain conspicuously on the ventral surface, and in that blue granules are detectable in the posterior zoöid region of *P. dorotocephala* very shortly after similar ones are visible anteriorly and before any are

to be seen at intermediate levels. As OH′ is reduced by addition of HCl the staining becomes more and more limited to the auricles and tip of the head (pH = 6.4), and finally at pH = 4.8 stained tissue cannot be found anywhere even after hours of exposure, when the acid itself kills. Probably for this reason animals stained very poorly in distilled water solutions of the dyes, for its reaction was about pH = 6.0. As a rule this species dies from the toxic effects of the distilled water before more than the few most sensitive parts of the head have been stained. In fact it is extremely difficult to get any basic stain at all into some specimens of oligochetes and protozoa when taken from an old, very acid culture. Sufficiently acid media apparently reversibly destain some vitally stained protoplasms to a certain extent; in many cases m.b. passes through a green or a more or less decolorized state. How much all these results are due to influence of the H ion concentration on dissociation and rate of diffusion of the dyes and how much to the alteration of membranes and deeper tissues is yet undetermined.

Susceptibility of all parts to a basic dye is usually increased by a definite alkaline reaction in the medium. In planaria the disintegration of the head is followed at once by the disintegration of the posterior zoöid region—an order which is not often followed if the reaction be acid. Alkalies seem to sensitize certain parts of the organism so that non-lethal concentrations of either the alkali or the basic dye combine to become lethal. It has been reported that alkalies increase direct susceptibility and acids indirect susceptibility (1), (4). But this fact should not be confused with another, namely, that an *inner* acid reaction probably increases suceptibility to basic dyes (6); both of these facts are consistently interpreted in the discussion.

Neutral salts markedly retard or actually prevent staining of all parts of planarians with m.b and other basic dyes. In this, $CaCl_2$ is more effective than NaCl. It is surely significant, however, that salts and hydrogen ion facilitate acid dye action.

Data in detail. The bulk of the data deals with the effect of m.b. on diverse species, and the results obtained are described for this dye with each form, frequent notes being added for other basic and a few acid dyes, when peculiarities or divergences in action were observed.

Planaria dorotocephala. This flatworm was singled out for particular study with the dyes because it was readily available and especially because it had been extensively used in the same laboratory for similar studies, and many data were already at hand of much value for comparison. Previous work with this had shown that individuals over

5 mm. are composed of a large anterior zoöid and of one or more smaller posterior zoöids not morphologically differentiated but clearly distinguishable physiologically (1). With these in mind attention was devoted chiefly to the relative speed and intensity of staining of different levels along the axis and to the time of disintegration of these different levels.

Staining gradient. Immersed in a solution of basic dye, planarians do not stain uniformly and simultaneously even throughout their surface area. Certain points of election are from the first visible. In general

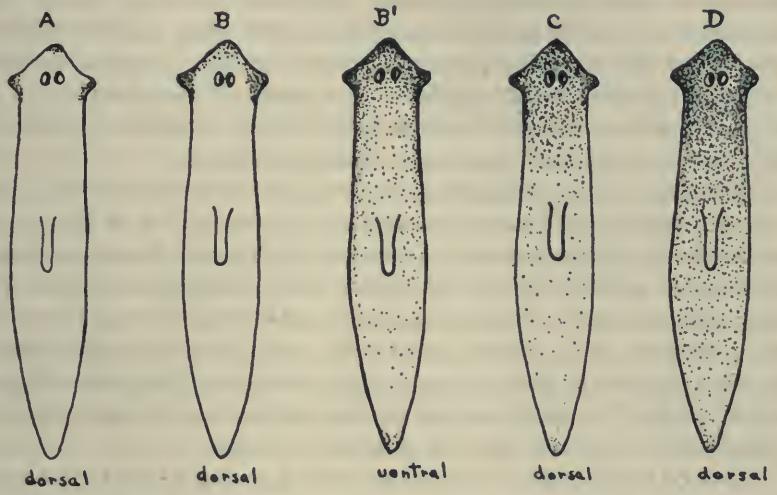

Fig. 2. Stages of staining of *P. dorotocephala* with methyene blue (and many other basic dyes) to show sites and relative intensity of coloration: *A*, stain in sensory lobes chiefly; *B*, spreading along margins of the head, and *B'*, same stage in ventral view, stain extending much farther than in *B*; *C*, and *D*, continued deep staining in more posterior levels. In alkaline media or with very young worms the stain shows early also in the posterior tip.

the first staining occurs within a few minutes or hours in certain parts of the epidermis. This order of such staining (fig. 2) is, practically without exception, first the lateral auricles, *A*, then the tip and later the ventral surface and margins of the head, *B*, *C*, continuing posteriorly thence until the whole animal excepting the proboscis is distinctly bluish, *D*, and finally quite blue-black externally. By use of diluter solutions and removal of specimens to clear water from time to time at appropriate intervals a series is obtained showing stained areas extending progressively backward and intensifying. At an early stage the animal is

quite distinctly "cyanocephalous" and is much given to moving the head in constant exploratory movement, or holding it stiffly erect. The ventral surface of the head takes on and retains a deeper coloration than the caudal parts and the dorsal surface, this difference persisting through further staining until shortly before cytolysis begins. In concentrated solutions the staining becomes more equalized throughout, and the initial differences are less evident. Curiously the proboscis remains strikingly uncolored even to the last in many dyes unless treated in a special manner to induce staining. In later stages of deep staining the anterior end is rendered more and more inactive, flaccid and unresponsive, and the part previously held erect drops under the ventral surface and the remaining parts roll or coil dorsally at ends and at the margins, in a characteristic fashion. If stimulated the animals may yet unroll and glide stiffly with the posterior cilia or muscles, but the uplifted anterior end is not touched to the substratum.

Teasing and close examination show the dye to be located in certain stained droplets and granules as well as to a less extent in the ground substance, first of the superficial cells and later in the deep-lying tissue. As a rule the stained constituents prove to be chiefly globules of an ever increasing size and number, occurring singly or several together inside larger globules of a bluish liquid. In m.b. made strongly alkaline the stain appears to pick out and color a number of irregular cells with central nuclei (?) situated on the ventral surface; no attempt was made to localize this stain by study of prepared sections.

Repeated tests made in various ways by exposing to H_2O_2 planarians stained only cephalically gave no indication of there being any colorless leucobase present but invisible in unstained regions, where it might conceivably be reduced. In fact, as Ehrlich found for m.b. in nervous tissue (7), the expectation would be that regions of high oxidative metabolism would reduce dye compounds to a leucoform more rapidly than the less active parts here left unstained.

Injured loci, wherever situated, take up basic stains considerably in advance of any uninjured parts: fission planes, either freshly or recently exposed or after the ends have contracted down and begun healing and reconstitution, exhibit a similar precocious coloration. There is no observational evidence for believing that simple exposure of or removal of a membrance from interior substances will promote immediate staining; the increased staining is such as would be expected to proceed from the stimulation of injury or the higher metabolism of contracting ends. It is interesting also to note that regenerating heads,

however translucent and apparently devoid of "density" and of differentiated structural material, yet stain easily and relatively deeply.

Previous killing by slow heating or by alcohol allows stains to flood in rapidly at all levels. Only in life was the staining gradient with basic dyes obtained.

Disintegration gradient. As bilaterally symmetric animals, flatworms possess three axes: the chief, antero-posterior axis, a ventro-dorsal one, and a medio-lateral one in the horizontal plane. Each of these axes should theoretically be represented by a gradient in metabolic or protoplasmic condition, such that a region of highest rate might be distinguished by its more marked susceptibility from other regions of lower rate.

As has been stated, there are low concentrations of stains which will stain without producing lethal effects anywhere. Once this minimal concentration is passed it is only a question of time when disintegration will set in. After the necessary toxic effect has been produced, the epidermal cells of the anterior end along the auricles and tip and ventral surface of the margins of the head assume a swollen and edematous aspect, loosen up from each other, lose their coherence and their original structural orientation, and scatter in small shreds, clumps and spherical masses, usually as small as the globules or granules composing the protoplasm. A disintegrating area decolorizes somewhat by extrusion of the stained constituents, so that the disintegrating portion is often sharply contrasted with the intact blue portion, as a loose, white, felt-like, downy tuft.

The order of disintegration of the epidermis and body wall (fig. 3) is most significant, since it affords an excellent readily visible demonstration of all of the three gradients believed to be present in the outer layers of the triaxiate organism. *a.* The zone of decoloration and of disintegration begins invariably on the auricles and tip and margins of the head, and proceeds slowly caudad. The disintegration belt is constantly shortened in front by detachment and loss of granules and droplets, and lengthened behind by the incorporation of more sound tissue into the disintegrating zone, which finally reaches the extreme posterior end. The belt immediately behind the disintegrating level is strongly contracted as in the sphincter-like closing of any injured part. Meanwhile the attitude of the head, margin and body is as described above. Parts left intact usually will exhibit some movements upon stimulation; only the more posterior levels retain power of adhering to the substratum. For a few minutes preceding initial

disintegration the individual passes through a stage of rhythmic movements; the wave proceeds postero-anteriorly—the posterior tip extends to its maximum caudally and then widens and shortens from the caudal tip forward, as if in attempting backward movement of an avoiding reaction. In moderate concentrations these rhythms may reverse in direction, several times alternating from posterior-anterior to anterior-posterior. *b*. The ventro-dorsal axis is also clearly indicated. Whitening and dissolution of tissue usually extend caudad more rapidly on the

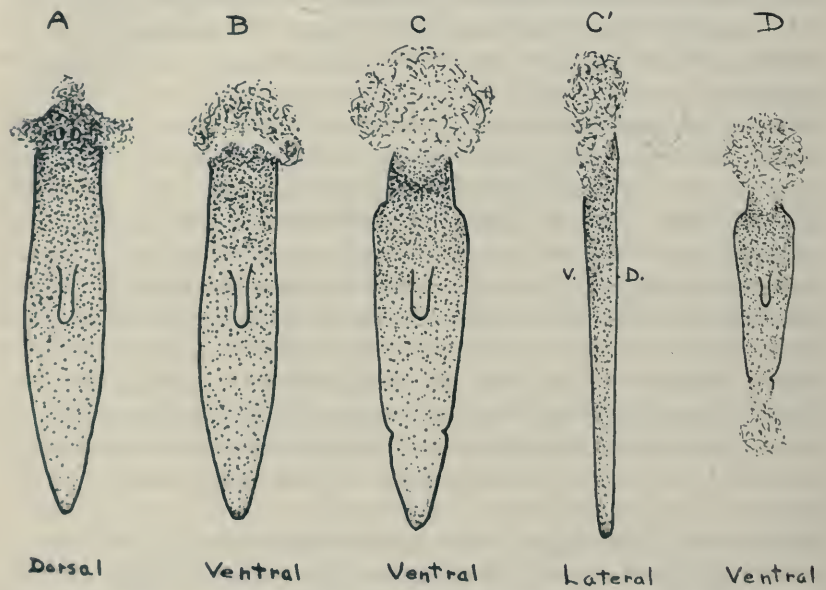

Fig. 3. Certain stages of disintegration of *P. dorotocephala* in methylene blue, showing correspondence with stages of staining of figure 2; and also the frequent precedence of ventral over dorsal disintegration, *C'*, and of median over lateral disintegration, *C*; *D*, and *D* the posterior disintegration of younger worms, or of any worm in a definitely alkaline medium of the dye.

ventral surface than on the dorsal (fig. 3, *C*). This more rapid advance of disintegration ventrally is obvious from the time the first tissue is lost under the head and is still noticeable posteriorly; in fact in many cases the ventral surface has entirely disintegrated when the dorsal parts of the posterior end are still intact in the form of a carapace-like shield of tissue, which is the last to display irritability and to disappear. *c*. Even the median-lateral axis is often demonstrable. Disintegration of the ventral surface does not usually advance back-

ward on an even transverse front. Often, and especially in high H ion concentration, disintegration of the epithelium is most rapid in the midline, so that the decolorized and cytolyzed area pushes back in a wedge shape and the living tissue is cut out to show a V-shaped front with two lateral arms projecting forward. In dorsal view the picture is more variable, and toward the posterior end the line of disintegration becomes more transverse.

In younger animals the posterior tip commonly disintegrates soon after the head. In more alkaline media all animals disintegrate thus (fig. 3, *D*). In no case was there any early loss of tissue at the anterior end of the second zoöid with basic dyes. A recent fission plane shows early disintegration corresponding to its early deep staining.

With dilute cyanide and various anesthetics Child obtained first disintegration along the margins, at the posterior tip, and dorsally at the anterior ends of intermediate zoöids. These results could not be duplicated with any basic dye or with acids, but were approached closely by use of alkalies and some acid dyes, as alizarin blue S (fig. 4).

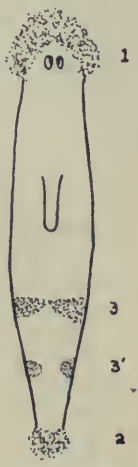

Fig. 4. An early stage of the disintegration of *P. dorotocephala* in alizarin blue S, and acid dye. Large specimens commonly show disintegration in the order *1, 2, 3, 3'*, or *1, 2, 3', 3*.

Other basic dyes differ from methylene blue chiefly in the degree of their irritating and toxic effects and in minor details of their staining. Naturally those with colors differing widely from the natural yellow brown pigment are most favorable for study of penetration of the dye, but all lend themselves to use as agents in susceptibility work—neutral red, crystal violet, victoria blue, magenta red, janus green, toluidin blue. The last two perhaps best show early staining of the posterior zoöid region.

Acid dyes—eosin, erythrosin, trypan blue, methyl blue, water blue, berlin blue, acid fuchsin, congo red, and many others—were tried in neutral solution but none became visible within the living animal even after hours or days. Only a few were toxic enough to kill. After death the dyes passed in but were easily washed out again. The action of alizarin blue S has been mentioned above. In more acid media the acid dyes are more effective, but here the acid effects seem to be predominant.

Planaria velata. In all essential respects this species resembles the above. Basic dyes penetrate and become visible within the proto-

plasm first in the truncate end and anterior margins of the head, and only later are to be seen at more posterior levels. *P. maculata* is even more similar to *dorotocephala*.

Phaenocora agassizi. This small white transparent rhabdocoel with large cilia was first met with and collected in abundance preying in a thriving ameba culture. On account of its small size (4–5 mm.) all observations were made under the microscope. This form is of some interest, for an individual is composed of a single zoöid and shows a simple steep main gradient. This gradient may be demonstrated with basic dyes in several distinct ways:

1. The staining gradient. Placed in 0.01 per cent m.b. the animal takes up the stain in a clearly differential fashion along the chief axis. A clear hyaline apparently structureless layer over the entire surface

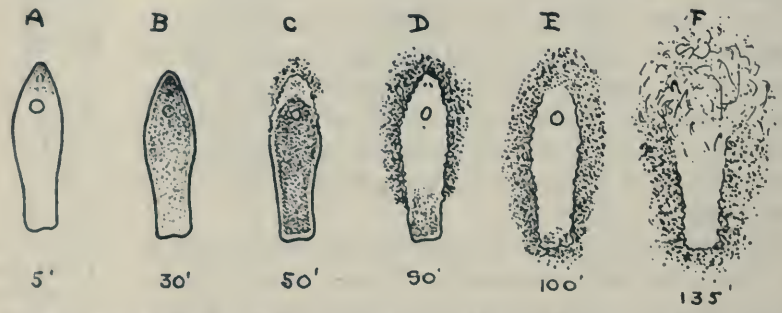

Fig. 5. The anterior-posterior gradient of staining *A*, *B*, *C*, of decolorization *C*, *D*, *E*, and of disintegration *E*, *F*, of *Phaenacora agassizi* in 1 per cent methylene blue.

remains relatively unstained. Almost at once the underlying tissues begin to stain around the sensitive point and edges of the reddish pigmented anterior end. It should be noted that this part lies well in front of the pharynx opening, contains no portion of the alimentary canal, and that no stain has yet become visible in the pharynx itself. The dye may be seen penetrating farther and farther caudad until in about 30 minutes its presence is indicated at the posterior truncate end. At this time the color differential is well marked; the pointed anterior end exhibits the first and the most abundant large blue granules which are progressively fewer posteriorly. Other concentrations give the same order of staining. The intensity of coloration continues to increase but the difference antero-posteriorly is never lessened until death changes are evident (fig. 5).

2. The gradient of extrusion of the stain. In about 40 to 50 minutes after exposure to 0.01 per cent m.b. the uncolored superficial layer in front of the pharynx assumes a somewhat bubbly outline as the cuticle is raised in small blebs. The edema or swelling in this zone doubtless implies changes in permeability and more or less local injury preluding death of the part, for closely following upon these alterations of state of membranes and tissues there ensues a conspicuous expulsion or escape of bluish granules and spherical clumps of cell material leaving the region without stain or pigment but surrounded by a blue halo or corona. The zone of extrusion slowly progresses backward and reaches the posterior end 45 minutes or an hour later.

3. The disintegration gradient. Immediately following the loss of colored particles a dissolution of structure sets in, manifested by further aggregation, clumping, swelling and lack of cohesion, fading away of the limiting epidermis and ultimate disorganization into semi-fluid transparent droplets or rounded granular masses. As superficial structures disappear and dissolve internal parts swell and push out. By the time colored particles are first thrown out from the intact posterior end, the anterior end is already disintegrating. At $2\frac{1}{4}$ hours disintegration has completely obliterated anterior structure and has been carried well toward the caudal end, the sound tissues being demarcated always by a sharp and well-defined boundary. Apparently in all cases the decoloration as well as the breakdown of tissue, once begun, is more rapid and simultaneous posteriorly, as if the gradient of susceptibility were more level and uniform there, though steeper anteriorly. That disintegration follows close upon death is indicated by the continuance of the ciliary stroke to the moment of disintegration when the beat becomes feebler and crawling and finally comes to a full stop.

Dalyellia (Vortex) viridis, also stains expels stained particles, and disintegrates in a definite anterior-posterior order.

Bothrioplana alacris? A small white rhabdocoel, evidently with triclad affinities, was collected from a temporary spring pond, but the sudden failure of the material left its identity uncertain. In 0.002 per cent m.b. staining begins definitely anteriorly, attacking the tip and especially the ciliated pits on the margin of the head. In 20 minutes the gradient is marked; at 30 minutes posterior parts are not yet stained, except sometimes at the extreme caudal end. In 40 minutes disintegration starts in ciliated pits and margins of the head and extends backward at such a pace that the anterior half of the

animal is removed in 55 minutes and the whole body scattered in 1½ hours.

Stenostomum leucops. As collected in April and May in indoor cultures this form consisted largely of chains of from 2 to 5 zoöids, but there were individuals in nearly all stages of fission and regeneration. Since a relatively high degree of differentiation (ganglia, ciliated pits, pharynx, etc.) is attained before zoöids separate from the chain, *Stenostomum* should be contrasted with planarians in which the posterior part is removed in a much less developed state.

A posterior zoöid of a 2-zoöid animal recently divided shows, if any, only a weakly developed secondary zoöid so that the gradient is simple and straight. The stain becomes visible first in the ciliated pits, then extends superficially to other more posterior levels. It finally seems to strike deeper and reach the ganglia underlying the pits, the other nerve structures, and sense organs around the mouth. Disintegration follows in 1 to 2 hours in 0.02 per cent m.b., usually before much stain is detectable posteriorly. Around the pits and over the main nerve masses the tissue swells even to rupturing, the protoplasmic masses taking deep stain when thus exposed. Hence in this case the disintegrating part is bluish. The ventral (oral) surface may disintegrate somewhat more rapidly than the dorsal surface.

An anterior zoöid recently detached from its posterior one stains and disintegrates at both anterior and posterior ends; the posterior end being both stimulated and exposed at the point of separation. Sometimes the fission end is lost well before the anterior end. In practically every instance disintegration of the anterior end follows the rule of midventral precedence over lateral and dorsal parts. In very acid media only the pharynx wall stains—in a sort of a network.

In an intact 2-zoöid animal the anterior end stains and disintegrates first; the new-forming anterior end of the second zoöid follows next in order, more rapidly if well formed, only after a time if not manifestly differentiated. A region where a fission septum is forming or where fission is taking place stains not only behind but also, and fully as much, in front of the septal plane. This region is doubtless subject to marked stimulation attendant upon the stresses and strains of the more or less violent separation which the dyes tend to induce. Individuals with more than 2 zoöids also tend to break up into discrete zoöids, but when the animal remains compound the zoöids stain as independently as in the 2-zoöid specimens.

Paramecium caudatum. Among protozoa *Paramecium* and *Dileptus* were chosen on account of their commonness in infusions, their elongate and axiate form, their comparatively exposed and uniformly ciliated surface, and the position of the oral aperture far back from the anterior end.

Most coarser stains, both basic and acid, are taken in and segregated in or near the food vacuoles of *Paramecium*, but m.b. and many other dyes of high visibility and marked color contrast also enter and stain somewhat diffusely even the less granular parts of the cell. With these agents, at 0.001 per cent or more, it is seen that individuals from actively dividing cultures show a distinct deep staining first in the extreme anterior end (fig. 6), soon concentrating below the ectoplasm in the outer endoplasm, in which the color spreads gradually backward; meanwhile the food vacuoles store much dye and collect posteriorly. In any effective concentration of the dyes the animals commonly

Fig. 6. Order of staining and disintegration of Paremecium in methylene blue.

reverse the direction of their swimming, or alternate in vigorous forward and backward movements, until they become sluggish and finally come to rest when they are heavily stained (except for the nucleus).

Shortly there occur changes in the gross appearance of the cell—the surface contour becomes more spherical, and the cuticle with its patterned markings, the cilia, etc., is lifted off the underlying parts and often ruptured, as if by inner swelling and the accumulation of a vacuole-like blister of fluid in some portion of the anterior end (not usually over the contractile vacuole). The fluid appears to force more and more of the more solid central contents posteriorly until the anterior portion of the cell has been practically emptied except of liquid while the caudal end is dense and crowded. The ciliary strokes cease soon after the outer layer is raised up, and always in the antero-posterior order, with many minutes intervening between their cessation in front and behind. It should be noted that only late in this process does the nucleus become deeply stained—a certain sign of lethal exposure.

This description applies quite generally for other basic dyes and for other typical ciliates, as *Stylonychia*, etc. Budgett (8) described antero-posterior dissolution of *Stylonychia* as a result of lack of oxygen, addition of KNC, pilocarpine, etc., and Child reported a somewhat modified gradient in several ciliates with KNC.

Dileptus gigas. This very large and elongate ciliate also reverses its direction of progression in the irritating dye solutions, until depression and paralysis ensue. In low concentration some basic dye is ingested and stored in the vacuoles, but in greater concentration little is thus taken in, and the animal gradually shortens and rounds out as it

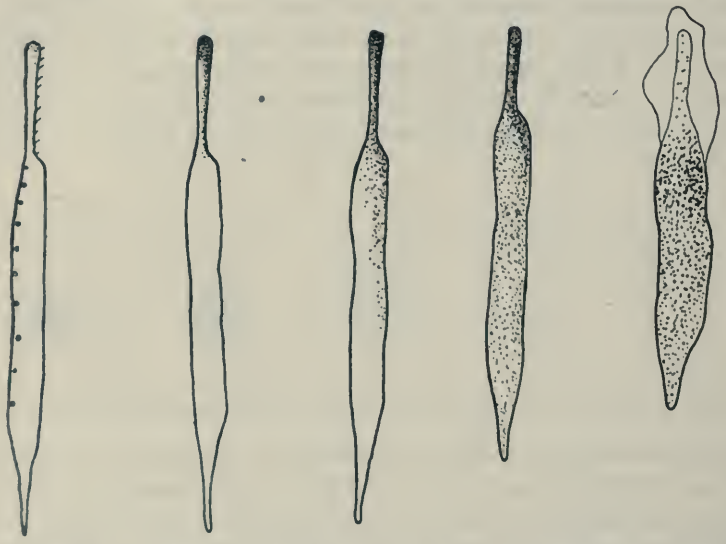

Fig. 7. The gradient of intravital staining and of disintegration of *Dileptus gigas* in methylene blue and many other basic dyes.

becomes quiescent. The stain enters first at or near the tip of the proboscis, especially along the row of large ventral cilia which extend back toward the mouth (fig. 7). Thence it continues to become visible further back in the middle regions and finally at the caudal tip itself. Disintegration occurs either slowly, or sometimes suddenly, with a loss of substance of the proboscis tip and base, of the oral region, and so on; or in some cases quite differently by a series of ruptures along the dorsal side opposite the many contractile vacuoles.

Hydra oligactis. Hydra is also instructive, in providing a case of a radially symmetrical animal where secondary budding may be followed in all stages (fig. 8).

A young hydra without buds, placed in a Syracuse dish, allowed to come to rest and attach for a time, and then covered carefully with m.b. (e.g., about 0.002 per cent), shows blue granules first in the ectoderm of the tips of the tentacles, on the mound of the hypostome, and on the body below the bases of the tentacles. The tentacles load up rapidly with stain, especially at their ends which soon surpass all other regions in their intense blue coloration. Dye meanwhile is detectable more and more basally upon the column and in a half-hour or more may be found everywhere in the ectoderm, excepting sometimes in portions of the base of the stalk. Nematocysts are commonly discharged instantaneously by strongly irritant dyes, and may be seen heavily colored throughout, either attached *in situ* or thrown out into the medium. They are practically always extruded first from the

Fig. 8. Order of intravital staining and early disintegration of *Hydra oligactis* in many basic dyes.

distal parts of the tentacles, and then more synchronously elsewhere. (Incidentally, this action of irritant basic dyes provides an excellent method for demonstration and study of the kinds, numbers and distribution of the stinging cells, especially of the smaller kinds shown only indifferently well by acetic acid and methyl green.) When disintegration occurs it also begins distally in the tentacles, which are slowly removed bit by bit down to their bases, after which the hypostome and adjoining oral parts are similarly broken down. Basally there is less order and regularity in the disorganization. While the stain was entering the ectoderm it was also accumulating in the gastrovascular cavity in an irregular fashion, chiefly at the bottom of the cavity, and somewhat orally reaching out into the bases of the tentacles. It is this fact, that the dye is taken in by the large mouth and made available to the endoderm, which helps to complicate so much

the later stages of disintegration in larger hydras. But even this cannot conceal the essential fact that in hydra a gradient exists from tip to base in the tentacles, oral to aboral in the column as has been shown by Drzewina and Bohn (9) with lack of oxygen, heat and chemicals, and by Child and Hyman (10) with KNC and dyes.

If a well-formed bud is present, it takes the stain and disintegrates in about the same order as the young specimen. With regard to the time of first staining of the bud and the parent there is fair constancy of behavior; usually the parent tentacles stain earliest, accompanied or followed soon by bud tentacles, and then by the body of parent and bud. The rounded or cylindrical elevation where a new bud is forming, even though it be but a proliferating rudiment, exhibits a considerable capacity for early local staining. A bud eminence thus stains prior to the adjacent parent body. Disintegration of tentacles of a large bud and of the parent occur at about the same time; a small bud without tentacles is disorganized after the parent tentacles but before the parent body of the adjoining level is attacked.

Neutral red gave evidence of a similar gradient: from tip to base in a tentacle, and from hypostome downward on the column. To congo red and phenol red, which can hardly be said to stain, and even to hydrogen peroxide, hydra displays a like differential susceptibility for it succumbs gradientwise in these agents.

Hydra differs from most animals used in that it will take up and concentrate a basic stain from solutions so dilute as to appear clear.

Among the Annelids a number of common fresh water species were used. For purposes of comparison and confirmation data on these forms has fortunately often been available from the work of Hyman (11), who gives an interpretive analysis of the process of regeneration and demonstrates and describes the gradients of susceptibility to KNC in many oligochetes. It will be obvious that, except in minor respects, dyes show the same gradients.

Aelosoma hemprichii. This small form was collected readily from mixed protozoan cultures. The conspicuous structures are: reddish oil globules imbedded in the integument, and, apically, the flat rounded sensory prostomium, a ciliated pharynx, and cerebral ganglia just anterior to the pharynx.

Both staining and the ensuing disintegration proceed down a gradient of a primary sort (fig. 9). In an intact animal without fission planes the stain (e.g., 0.005-0.01 per cent m.b. for 1 to 4 hours) shows first in the ciliated pits and thickened sensory epithelium of the rounded ventral

surface of the prostomium and in the oral epithelium, but is soon visible also in more dorsal areas above the mouth between the pits and over and in the two ganglia lying superficially in full contact with the epidermis. From the deeply colored anterior end the staining area (and disintegration later) is carried backward at first slowly behind the mouth and then in more rapid sequence through the more posterior levels. In this progress stain does not enter segment by segment but in continuous gradation. The oil globules appear to stain about equally rapidly at all levels. The ciliated pharynx and the oral parts of the intestine may draw in and accumulate quantities of the dye, and produce a local area

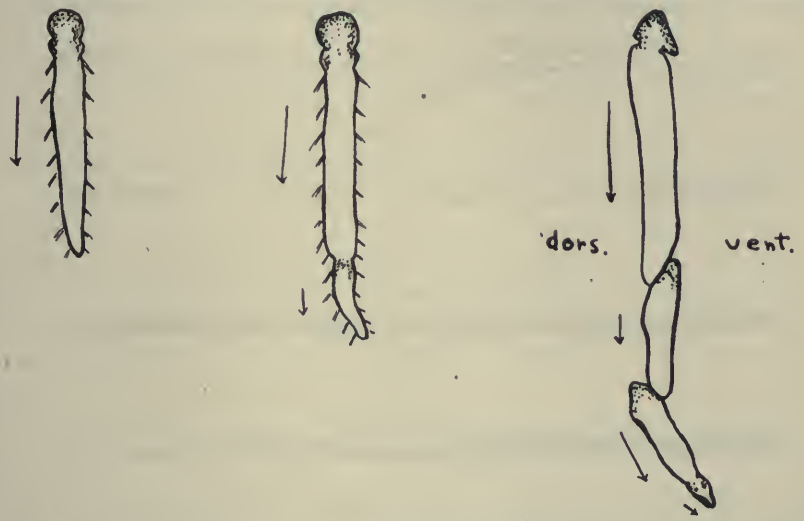

Fig. 9. *Aelosoma hemprichii*, showing order of staining of different specimens, composed of one, two or more zoöids.

of deep staining disturbing the simple gradient, but the first parts actually to stain lie wholly in front of the alimentary canal and out of communication with it. The posterior end stains early if a posterior zoöid has recently been removed.

If an animal possesses a marked fission plane, the stain enters in a definite ring of epidermis at or near each anterior end and concentrates, especially on the dorsal surface, behind the plane. The further course and times of staining are similar in both zoöids, which are ordinarily separated by the disintegration of the anterior end of the second.

Dero limosa. The important structures and their order of staining are shown in figure 10. In 0.01 per cent m.b. a sound specimen without

zoöids stains quickly in cutaneous portions, particularly anteriorly in the prostomium and ventral sensory buccal areas. An especially active ciliated gill region at the anal end composed of gills in a respiratory pit colors about as soon. Staining progresses slowly back from the head and more rapidly from the anal end forward. In this latter course it moves by segments or small blocks of segments, which stain first near the septa where, intersegmentally, a sphincter-like contraction takes place and forms a chain of blue bead-like rings, such a chain lengthening by additions from in front. In each segment the deepest mass of dye accumulates in ventral patches apparently corresponding in position to the segmental ganglia. The advance cephalad is soon met by

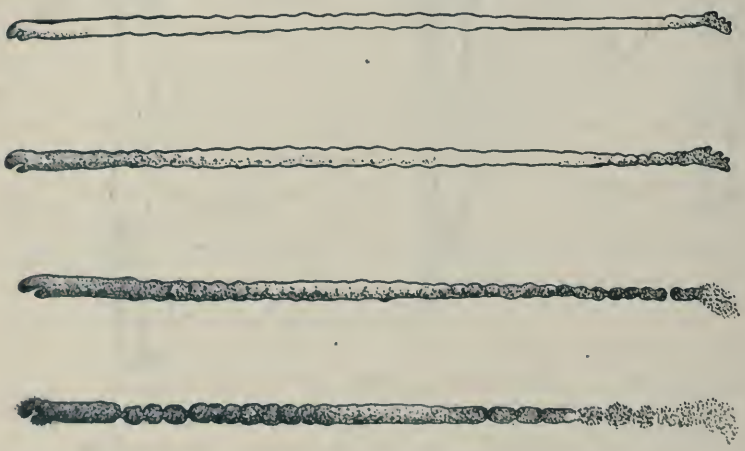

Fig. 10. Progress of staining and disintegration in *Dero limosa*, resembling generally that for most higher Oligochetes.

anterior staining moving caudad. In this and the higher annelids the cuticle seems to interfere with the penetration and fixation of the dyes, for in all staining is uneven at any given level and may wash out for some time after it has passed through the outer covering.

When two zoöids are present, the anterior stains the more darkly at first and in the antero-posterior order. A posterior zoöid stains less deeply, at first postero-anteriorly and later also from its anterior end back. As differentiation of cephalic structures proceeds in this zoöid these stain more and more quickly, finally equalling or even anticipating the anterior end of the first zoöid.

The first losses by disintegration are from the gill region and several of the posterior segments. Then follow the prostomium and the next

succeeding anterior segments. By the time the head goes the last 10 or 12 segments have been cut off one by one or in small groups. Intermediate portions may recover partially after losses from either end.

Lumbriculus inversitans. In 0.001 per cent m.b. epidermal structures stain chiefly at the anterior end and the caudal tip. The head stains quite uniformly along its length; it lacks septal divisions. Dye is soon visible more or less irregularly in intermediate regions, and edematous swelling occurs at corresponding points.

A part becomes pale blue on disintegration. The caudal third or more of large worms, beginning posteriorly, has formed a bead-like series of blue segments, as in *Dero*, by the time the head shows first signs of actual breakdown. The wave of further disintegration spreads backward from the head and forward from the anal end. Segment by segment the stain floods into the tissues and then is partly lost again in the subsequent dissolution process. Smaller and larger worms begin disintegration about simultaneously, but smaller ones may complete it sooner.

Tubifex tubifex and *Limnodrilus claparedianus.* Cutaneous parts, bristles, as well as the whole superficial nerve plexus take on stain early. In small, 1 inch specimens the anterior end colors first. In larger ones before the head segments stain perceptibly posterior ones have long since colored deeply and often broken off. In *Tubifex* where the cuticle is thinner posterior segments drop off one at a time; in *Limnodrilus* they detach in sections. Meanwhile the whole anterior tip loads heavily with stain. Prior to the sloughing off of segments the purplish stain seems to concentrate heavily along the lateral portions of the segments and then spread elsewhere; otherwise anterior rings stain most prominently at the bristle level and posterior ones in blue bands intersegmentally. Finally the dye concentrates ventrally in each segment. The last parts to stain are the dorsal portions of those segments somewhat behind the head. Rhythmic pulsations of blood vessels and alimentary canal continue regularly until slightly before the deep staining of the approaching death-point is attained.

In disintegration the parts swell greatly and constrict between segments. Usually the posterior half is lost by detachment of rings or groups before the head dies; even in 0.001 per cent m.b. 20 or more anal segments were dropped in $3\frac{1}{2}$ hours. Head and succeeding parts go next, the last surviving segments being a block about one-fourth of the distance back.

The chick embryo. The incubated egg was opened and immersed in a solution of 1 part m.b. powder in 5-10,000 parts of isotonic saline, previously warmed to incubation temperature and held at about that point for the course of the experiment.

The blastodisc, germ wall, and actively growing vascular area stain before the yolk or vegetal pole. The regions most sensitive to coloration are the formative regions: the edges of the uprising medullary folds in the anterior parts, and the sides of the neural groove posterior to the head fold, fading out more posteriorly until the level of forming somites is reached and back toward the anterior end of the primitive streak, where deeper stain is again met with.

On 3-somite chicks the medullary folds and head ectoderm are the most conspicuously stained portions anteriorly, and the end of the folds posteriorly, where some active growth and proliferation is taking place.

Five to seven-somite chicks stain deepest on the head, the intensity thence grading down in the region of formed somites and then gradually rising again in the extending medullary plate and folds. It certainly seems to be clearly possible to distinguish here a descending primary gradient (of differentiated structure) and a posterior ascending (growth) gradient, resembling generally that in annelids. At least the ectoderm of a 10 to 12-somite chick becomes a deep opaque blue-black in the head, which fades out to the end of the region of the closed medullary tube, whence it again deepens toward the posterior ends of the medullary folds. The trough of the medullary groove is comparatively lightly stained. The bulk of the stain accumulates at first only in the ectodermal layer, for this may be peeled off leaving the interior practically uncolored. The mesodermal somite regions, however, evidently show a somewhat similar gradation of superficial staining later, lowering from the head through the formed somites and rising sharply toward the posterior end of the region of developing somites and anterior to the unsegmented plate.

DISCUSSION

In at least three ways the intravital dyes might be expected to contribute to a solution of the problems of differential susceptibility and to a closer study of the real nature of a "dominant region" or active state. (1) The movements of their conspicuously colored particles may be followed more readily than those of most agents, their points and rates of penetration and sites and manner of storage noted, and the facts so

obtained correlated with the results obtained by the susceptibility method with the dyes and with other agents and conditions. (2) Alterations in penetration and storage may be studied: *a*, during different functional states; and *b*, with the dyes in media containing dissolved salts, acids and bases. (3) Use may be made tentatively of the physicochemical properties of the dyes and of protoplasm, as far as known, for analysis of the process of staining and of the manner of production of dye effects upon the organism. This applies to dyes, toxic and relatively non-toxic, vital staining and non-staining, as well to those undergoing some definite change (oxidation, reduction, or a change in color indicating reaction, or a change of state) as to those which apparently resist such changes. Obviously this work makes use of by no means all the possibilities of the method.

1. The susceptibility gradient with dyes. The general significance of the differential susceptibility existing along the structural axis of axiate organisms has been often and fully discussed (2),(11). In the light of the data derived largely from these previous studies with KNC, lack of oxygen, and narcotics, and from the quantitative determination of gas intake and output, there seems to be full warrant for the belief that, under conditions where death occurs at all rapidly, the relative susceptibility indicates more or less delicately a gradient in some slight differences in metabolic activity, associated in all probability with oxidations.

As described in the details of this paper, the numerous basic and rarer acid dyes which are toxic enough in nearly neutral solution to be distinctly lethal, produce disintegration beginning in cephalic parts and other sites of known markedly high metabolic rate—this disintegration agreeing, so far as the facts have been determined, in fundamentals with that for cyanides, etc., in the same forms. The chief divergence in results occurs in the lack of delicacy of the dyes in distinguishing the small differences detected by KNC. Even such dyes as m.b., which undergo oxidation and reduction changes, failed in the same way and were disappointing, inasmuch as at the beginning of the work it was thought likely *a priori* that they might give positive results. Helpful facts should certainly be obtained from the use of methylene blue and its leucobase in conditions where oxygen can be supplied or withdrawn at will.

The nature of the difference in susceptibility to dyes as compared with that to KNC, etc. is interesting and perhaps significant, particularly if compared also with susceptibility to acids and bases (4). It may be

fairly stated that in this matter and in general physiological effects basic dyes behave much like acids (HCl or acetic), and acid dyes much like alkalies (NaOH). Thus both basic dyes and acids produce in flat-worms a most rapid disintegration of the anterior end, often of the ventral surface as compared with the dorsal, sometimes of the midline as compared with more lateral parts; the anterior ends of the second and succeeding zoöids are not singled out for early disintegration; no matter how low the concentration and how slow the killing, disintegration, when it does occur progresses posteriorly; and finally, in any quickly lethal concentration the young and old individuals are nearly equally tolerant, the young being but slightly, if at all, less resistant than the old. Acid dyes, conversely, resemble alkalies in their effects, though seldom sufficiently toxic to be readily lethal except in solutions made considerably acid. Thus alizarin blue S causes most rapid disintegration of the anterior end, dorsal surface and lateral margins, and often of the anterior ends of the second and third zoöids; and young individuals are plainly much less tolerant than older ones; and in certain cases indirect susceptibility was observed.

These similarities of action had not been anticipated, in fact were discovered entirely empirically, but might logically have been expected from the likeness of the dissociation of these agents. The neutral salts of basic dyes hydrolyze freely in neutral or alkaline solution, and dissociate into a large colored basic ion, the cation, which moves toward a cathode, and the anion radical, e.g.,Cl', of a strong acid. Evidently *the large color ion, like the mobile II ion, predominates powerfully in effect over the anion.* On the other hand, acid dyes dissociate, if at all, into a large colored anion resembling hydroxyl ion in effects, and a less potent metallic ion like Na^+.

The influence of impurities, such as heavy metals, in these dyes deserves treatment, of course, but these did not seem to interfere with the action above described, which I interpreted as due to the dye itself, since all samples of numerous dyes from varied sources gave like effects. If acids are added to acid dyes or alkalies to basic dyes to increase their action the results are naturally neutralized and confusing.

2. Decolorization gradient. That a gradient of decolorization by extrusion or escape of dye and dye particles should be found in some forms, as many flatworms, is not really a new observation. Such loss of stain, like the loss of natural pigments many times reported occurs commonly when death is imminent, and may be taken to indicate that resistance to the passage of diffusing substance has disappeared, and

conditions of normal semi-permeability destroyed, or perhaps, in this case, rather that retaining surface layers have been broken and loosened by beginning disintegration, and allow escape of discrete particles, especially if these are under pressure from adjacent swollen tissue or from a cover slip. Recovery is, however, often still possible after a certain amount of this loss has taken place.

3. The staining gradient. Although a sort of coloration gradient had been reported by Child with potassium permanganate (12) and with indophenol (3), the field of true axial protoplasmic vital staining with dyes has been entered deliberately only in this and associated studies on algae and hydroids (13); hence the facts merit brief discussion here.

Any doubt of the general observation that with easily visible basic dyes, such as methylene blue, toluidin blue and many others, there is produced, as reported for the forms used, a color gradient corresponding approximately to the major susceptibility and metabolic gradients (as determined by HCl rather than by KNC), may be removed by a few simple but convincing experiments such as above outlined, with organisms consisting either of one zoöid or of two or more morphologically distinct zoöids. Generally speaking, the order of staining of parts corresponds to the order of their susceptibility to acids and to the basic dyes themselves. Slight differences of susceptibility as shown by cyanides are not usually indicated either by susceptibility or by staining with these dyes, but it is safe to state that, knowing the order of staining with basic dyes one can quite reasonably predict the general course of disintegration with most lethal agents, or knowing the relative susceptibility of parts, their relative staining times may be foretold.

Concerning the correct interpretation of these findings there may well be some diversity of opinion. Where so little is really known one may not safely become assertive or dogmatic. But I shall point out a few indications of the rôle of some physico-chemical factors underlying, conditioning, or associated with metabolic gradients, to this end invoking the aid of current doctrines. To attempt to apply the classic as well as more recent theories of vital staining, both "physical" and "chemical," may help to recognize, eliminate or evaluate the factors here concerned.

First of all, one may be assured that there is no simple gross anatomical explanation to account for the axial ingress of the stain. The mouth is seldom situated at the cephalic end of the staining gradient, and is often well back, e.g., midway in flatworms, and at the base of

the tentacles in hydra. The rôle of the alimentary canal as a path of staining is easily recognized, as in the cases of *Hydra* or *Aelosoma*. The first parts to dye are usually particles or globules in or subjacent to the epithelial surface and stain passes in through the surface not by any large aperture. Nor is there staining of some special tissue alone, though ciliated and sensory surfaces and nervous elements are oftenest first conspicuous. The gradient is easy and continuous, but need not be always antero-posterior, as differentiated structure would commonly be; in higher annelids the dye enters earliest at the ends, and later in the middle regions. Much the same limitations apply to a "density" factor. If metabolic activity leaves an accumulating residue of organized reserve or inactive stabilizing substance, then as differentiation proceeds this material might be laid down gradientwise and be stained accordingly; but a newly regenerated head, highly transparent, nongranular, and relatively undifferentiated accumulates stain earlier than adjacent posterior levels, and the staining particles appear identical throughout the axis.

In view of Ehrlich's demonstration (7) of the relation existing between staining capacity with methylene blue and the rate of oxidation in different tissues, the assumption was made *a priori*, that stain might enter equally all along the chief axis but become invisible in certain parts through transformation into colorless base by marked local reducing action. That such is not the case here is shown by tests with strong oxidizing agents, as H_2O_2, which fail to reveal, i.e. make blue, any such invisible dye base. On the contrary, there is every reason to believe that a greater power of reduction in deoxygenated water must be possessed by those most active parts, first staining in aerated water: and it seems likely that in deficiency of oxygen supply reducing power is a criterion of vital activity, while in abundance of oxygen staining with methylene blue is such a criterion. Certainly in well oxygenated media any local reducing action may be ignored entirely, especially since in most of the vital stains the colorless form does not exist.

The gradient may, of course, be "explained" and dismissed as due simply to differences along the axis in *permeability* of the membrane to dye particles. If by this term one implies some kind of ultrafilter in the sense of Ruhland (14), then it may I think be rejected, for the size of particles of basic dyes is increased in the same alkaline solutions that facilitate their penetration and the same is true for acid dyes in acid solutions (6). In any case the mechanism of alteration of permeability itself requires analysis.

For the popular Overton theory (15), maintaining that the entrance dyes, basic or otherwise, into nervous and other tissues is determined by their relatively high solubility in lipoids that collect at surfaces and phase boundaries, there is no support here. The theory obviously cannot hold both for the lipoid-soluble dyes (janus green, dahlia, neutral red, methylene blue, methyl violet) and for the similarly acting lipoid-insoluble dyes (toluidin blue, thionin, methyl green), all of which enter and exhibit strikingly like effects in the cases tested. The intravital stains are mostly basic, and many of them have been designated as "specific" nerve stains, but even for the nerve it is not the myelin sheath which is colored, but the neurofibrillae and Nissl bodies (16). Solubility in lipoids would appear to have no prominent influence in controlling distribution or effects of dyes as here described.

Ehrlich and his students, Fischel (17) and Goldman, contended that vital dyes react chemically with definite specific dye-receptors of large protoplasmic molecules, which receptors might conceivably concentrate in graded amounts along the axis and could thus determine and measure the relative affinities of various parts of the animal for the stain. It is hard to refute this view but many facts stand against it as stated in its original form: first, nearly all or all levels parts and tissues of the organism stain *finally* to approximately the same depth; it is merely a difference of time required to bring in the stain and make it visible, not of stainability or of amount of stain taken up ultimately. Second, the marked non-specificity of staining with the various basic dyes points to some more fundamental common property conditioning the reception of the stain. Chemically unlike dyes (thiazins, azo-dyes, etc.) (5), (18) often behave alike, and those of closest chemical relationship are as frequently opposing in effect. Further, practically any lethal agent or condition will produce a disintegration gradient of an essentially similar nature, differing only in minor respects-cyanides, narcotics, lack of oxygen, excess CO_2 or other waste products, salts of heavy metals, dyes and indicators, ions of electrolytes, high and low temperatures, and doubtless a great variety of other unfavorable conditions and of agents of no known or obvious chemical similarity or kinship. As to the dyes, the most prominent cleavage among them is not one of specific chemical constitution but that between acid and basic, a matter of reaction and manner of dissociation. Admitting the probability of some specificity in details of working of individual dyes does not warrant speaking of staining as a specific chemical process. There must be some widespread and less specific chemical or physical character responsible for the course of basic vital staining.

In the writer's opinion the staining gradient may well be due to the greater entrance and fixaton of dyes in certain regions corresponding to a graded difference in adsorption or combining capacity, itself based on metabolic activity. This conception is not inconsistent with the belief that continued entrace of the dye depends in large part on its power of combining with or being precipitated of flocculated by certain constituents of the cells, and that its accumulation is possible because more or less insoluble compounds are formed within (19).

Valid evidence as to the mode of fixation of the dye may be obtained from the study of the conditions necessary for staining of textile fibers, proteins, etc., *in vitro* and *post mortem*. Basic dyes, yielding electro-positive colored ions, form insoluble colored salts with many "acid" protoplasmic substances containing organic acids combined with strong bases, e.g., mucin, hyaline cartilage, nuclein, amyloid, casein, Nissl bodies, yolk material, soaps, etc., the dyes combining like metals to form a basic-dye-albuminate, etc.; but basic dyes will not combine with the more basic or neutral albumins, globulins, albumoses, histones, etc., except in alkaline solution. These latter substances, however, especially in acid media, combine readily, often with a precipitation, with free inorganic or organic acids and with acid dyes of all kinds, giving an albumin-acid-dye compound (20), (21), (22). Neutral gelatin combines with neither acid nor basic dyes; if made electro-positive it stains with and retains acid dyes (acid fuchsin), and if made electro-negative it takes up and retains basic dyes (neutral red) (23). In general, previous adsorption of acid or neutral salt ions tends to discharge and aggregate electro-negative colloids, and accordingly both *in vivo* and *in vitro* decreases basic staining and increases acid staining, while conversely, alkaline solutions favor basic staining and diminish acid staining (5), (24), (25), (27).

These suggestions by way of an electro-chemical view of staining are supported by the facts of vital staining. Methylene blue and neutral red form insoluble dye-tannate compounds with tannic acid of the sap vacuoles of *Spirogyra* (28); neutral red forms a soluble red compound with some organic acid in *Elodea;* while in animal cells, as in Paramecium and in Echinoderm eggs union is made probably with some lecithoprotein (29). From recorded and new data Von Moellendorf (27) concludes that basic dyes react with natural acid colloid (anion) constituents of cell protoplasm in the same manner as they react with acid dye ions either *in vitro* or stored previously as granules *in vivo;* but basic stained protoplasm cannot thus combine later with acid dyes!

Matthews believes that basic dyes stain because they form some insoluble compound (salts, esters, etc.) with the "proteinate," "lecithinate," amino-acid, cholesterol, fatty acid, and other similar ions of protoplasm. The compounds may be regarded as weak surface chemical combinations quite insoluble and inert chemically, usually but not always highly stable, sometimes slightly dissociating and partly reversible.

That animal protoplasm is usually electro-negative is attested by both electrical and physiological observations; under ordinary conditions and in ordinary slightly alkaline media the proteins and lipo-proteins of which it is composed, having low iso-electric points, dissociate with negative charge (5), (30). Electro-negative colloids behave electrically like anions of an acid, and anions combine with or adsorb positively charged metal or basic dye cations, but not acid dye anions, which wash out leaving no stain. Even a prolonged immersion in an acid medium does not commonly, but may sometimes, suffice to bring about acid dye staining. In the exceptional cases where colloids are positively charged, as in hemoglobin of erythrocytes, perhaps in many plant cells, and apparently in some leeches, it is the acid dyes that really stain. In extreme acidosis also tissues may stain vitally with acid dyes (31). From this view the likeness of the effects of basic dyes and acids, both with predominant cations, is to be expected.

If anions or acid radicals are important requisites of basic staining, then there would need be a graduated production of such anions along the axis to account for the staining gradient, as well as a greater abundance of them in certain tissues, as nervous tissue, and in active parts generally. A constant, and in life unfailing, source of these anions may be sought in the katabolic processes, which yield acid products always on the whole preponderating over the ammonia produced. From split products an increased number of molecules results which, like amphoteric substances in the alkaline interior of the cell, dissociate as acids with a maximum number of anions and a certain number of H ions. This would not necessarily result in any actual acid reaction even locally; one can only speculate as to the disposal of the H ions, whether by neutralization, or by rapid escape outward, or by formation of a Helmholtz double layer; rapid liberation of H ions may produce the galvanometric electro-negativity of the active part (32).

It is interesting to note what has been taken as a curious old observation, that only the color ion of a basic dye is adsorbed, while the Cl ion is left in the outer solution (5). Similarly only the H ions of an HCl solution are absorbed, according to Gray (33), who says that the

charge of the Cl′ outside is satisfied by outgoing K ions, which are themselves replaced by H ions. It seems likely that basic dye ions likewise take the place of H ions, providing these are able to make their escape into the outer medium (as in neutral or alkaline media), but if the H ions are unable to escape (as in acid solutions without, or with acid reaction within the cell) they would block the entrance of the dye ions. For there is considerable evidence that if reaction of the cell actually becomes acid it no longer stains with basic dyes (6). In general it is sufficient if one of the results of rapid metabolism is a correlative net increase, momentarily at least, in negatively charged ions, which constitute the basic staining substance or condition. Naturally if the anions are formed by dissociation of amphoteric proteins as acids, their production should be reduced by acidity and facilitated by alkalinity, by abundant oxygen, rapid diffusion of CO_2, etc.

The rate of entrance of a dye may be controlled not alone by capacity to form compounds within, more or less depending on metabolic activity; conceivably a greater water content of active parts may aid in more rapid diffusion, or katabolic acids may lead to a more aggregate or more swollen condition, which may stain more rapidly. And there are doubtless other unanalyzed factors in the graded permeability along the axis; in the next section high permeability to dyes will be seen to be practically always associated with states of stimulation and activity.

I believe the points to be not witnout significance that all of the dyes of whatever chemical consitution and however varied the details of their staining pictures, were always in substantial agreement as regards the parts first stained as well as in the final staining gradient; that the dye first become visible at the same definite loci (e.g., the auricles of *Planaria*), whether staining exactly the same substance or not; that these loci are practically always the loci of greatest general susceptibility; that all of the really successful vital stains were basic, while the acid dyes were as generally signal failures; and finally that this staining is apparently dependent upon metabolism, since previously killed animals show no staining gradient.

4. The essential similarity of protoplasmic condition in the dominant region, a stimulated region or condition, and in a fertilized egg. Of more than ordinary interest are the variations in staining in different physiological states, particularly that of excitation. For instance, recently traumatized regions, stomach ulcers (34), and healing wounds are characterized by a state of enhanced metabolic activity, increased res-

piratory exchanges, by weakening of the surface layers, and accordingly are stained easily and strongly.

There is in the literature considerable direct evidence that capacity for taking and holding basic stains varies to some extent with metabolic and respiratory activity. For gland cells the facts are fairly complete and all in full agreement. Asher and Garmus (35) and Garmus (35) showed by direct continuous observation that gland cells of the nictitating membrane of the living frog take up much more vital dye of all various degrees of solubility in lipoids (m.b., neutral red, rhodamin, bismark brown, toluidin blue, thionin) when stimulated to great functional activity by pilocarpine than when left unstimulated, and more when left unstimulated than when function was depressed by atropine. After injection of pilocarpine the secretory granules, etc., were more quickly colored, and with time their coloration became strongly intensified. After local or general atropinazition coloration began very late and never became more than weak, although the cells were as richly granulated as resting or normally functioning cells. The difference could not have been due, therefore, to a greater secretion, but must have been brought about, according to the authors, to an alteration in the permeability of the membranes. Keleman has since shown (36) that pilocarpine increases respiratory exchanges and CO_2 production as much as 10 per cent and increases CO_2 of both arterial and venous blood, while atropine diminishes these below normal. Pilocarpine also raises the relative galvanometric electronegativity of gland cells stimulated by it.

For nerve tissue a similar condition apparently obtains. Bethe (16) discovered that during and shortly after the passage of a polarizing current through a living nerve the neurofibrillae lose absolutely all power of primary staining with methylene blue or toluidine blue at the anode but increase their normal capacity for the same dyes at the cathode. After recovery is complete the staining polarity disappears. These physiological changes exactly parallel the electrotonus changes: decreased irritability and conduction at the anode (anelectrotonus) and increased irritability at the cathode (catelectrotonus); that is, primary stainability varies directly with irritability and conductivity. Bethe came to believe after some experimentation with alkalies, distilled water, narcotics, etc., that at the athode there is an increased affinity of the neurofibril protoplasm for a "fibril acid substance," which moves toward or otherwise becomes more abundant and firmly attached at the cathode pole and there makes the fibrils more stainable. Frequent stimulation, strychninization, and a relative predominance of

katabolic processes over anabolic gives a cathodal appearance to staining fibrils; prolonged rest or excessive stimulation gives an anodal appearance. In death and deep narcosis no polarity is produced. According to the view here suggested the cathode may be conceived as stimulating the production of metabolic acids and attracting and withdrawing from the fiber positive ions, in whose places may be substituted the basic dye ions. Neurologists have repeatedly found that an increased amount of basic staining occurs, e.g., in Purkinje cells of the cerebellum and other motor cells, after first stimulations, but that with excessive stimulation ending in fatigue and exhaustion the staining substance or condition vanishes and less and less staining occurs. Pollock and Cluney (37), commenting upon the results of intravital staining of brain cells of mammals with various dyes introduced into the blood stream or under the meninges, say that "any procedure which increases metabolic activity of cells insures a greater degree of intravitam staining" or ingestion of trypan blue.

Matzumoto (38) reports that very dilute (0.00001 per cent) neutral red or nile blue sulphate after 10 hours to 4 days stains characteristic granules of corneal epithelium of the frog. He notes a general parallel between activity of cells and presence of stain, the deeper basal swollen and active cells (which are more acid, according to Unna (39)) staining in many granules of all sizes, while there is progressively less staining in more superficial cells, and none at all in the flat polygonal cells of the surface.

There are many points of likeness, also, between the processes of fertilization (initiation of cell division) and of stimulation. Arbacia eggs shortly after either natural or artificial fertilization show a two to three fold output of CO_2 and of heat, a vastly increased O_2 consumption, a diminished electrical resistance (as in working striated muscle (40), (30)), an increased permeability to salts, water, natural pigments, and an enhanced stainability with methylene blue and neutral red (41). The most deeply staining individual eggs divided first.

In the light of new observations and relevant literature basic vital staining of different cells and tissues and of different parts of an axiate organism may be understood tentatively as indicating differences in staining condition more or less paralleling metabolic activity, which produces substances with anion radicals capable of combining with positively charged ions. It is yet impossible to clearly dissociate this combining capacity from accompanying and secondary (?) differences in water content, ion content, and especially in "permeability." It has

become increasingly obvious as this work progressed that a sharp distinction could hardly be drawn between chemical reactivity, combining capacity, and permeability; and indeed a semi-permeable membrane is not necessarily a definite, physically detachable, primarily static and unchangeable structure, but should be and commonly is (5), (30), (42) regarded as an integral part of the protoplasm, undergoing all of the changes characteristic of living substance, and hence showing with it similar modifications by agents in the medium as well as its alterations during functional activity. In fact concentrated and exposed surface protoplasm almost certainly participates in these changes even more quickly and completely than less available, remoter, interior parts, and the preliminary effect of an agent or physical condition (in compounds produced, altered or destroyed, or in aggregation and solution effects, etc.) may well facilitate or retard subsequent action and penetration of the agent, or effectiveness of the physical condition. Thus surface metabolism and condition may control general metabolism.

Semi-permeability is probably maintained by metabolic activity. It is interesting to see that organisms killed by heat or alcohol stain quite heavily in a fraction of the time required by a living individual, and in the dead animal no gradient appears, but intake of stain is quite uniform throughout, special sites of election being to all appearances absent.

As staining and susceptibility follow each other closely in their respective courses, it is readily conceivable and probably true that, though cells apparently uninjured and with unaltered membrane take up the stain, a state of excitation, such as we must suppose exists in a dominant part, amounts substantially to a mild injury; that there are all degrees of injury up to death itself; and that staining, like permeability, increases in general after injury and as death approaches.

A region or state of dominance in an axiate organism is thus characterized by possessing distinct differences in susceptibility, in regenerative capacity (2), in respiratory activity (3), (43), in electrical potential (32), probably in catalase content or activity (44). Evidence of differences in rate of vital staining or in permeability has been presented in this paper. In most of these properties the dominant region resembles any metabolically active or stimulated part. Whether it resembles an actively functioning part in certain further respects is yet to be determined. An examination for high water content may be made by analysis; heat production may be measured in favorable forms by some thermocouple device; and electrical conductivity may be quantitatively determined.

In view of the exact and extended parallels which may be drawn between the physiological state of the dominant region and that in any stimulated region it is helpful to conceive of the dominant region in general as a portion in a condition of more or less permanent tonus or continuous partial stimulation, more highly irritable and more quickly responsive than less active parts, and therefore as always activating or stimulating subordinate levels. It responds to stimulation after a briefer latent period, or period of restitution and recovery. Since its activity is but little arrested after responses it is relatively non-fatiguing (like nerve as compared with muscle), and because of these attributes it possesses to a greater degree than other parts automatism and spontaneity, the capacity of "initiating" impulses.

SUMMARY

These researches were undertaken to determine further the nature, characteristics and mode of action of metabolically active parts or tissues, especially those of the "dominant" (cephalic, apical, anterior, etc.) region, chiefly with the aid of a representative series of stains.

The methods employed were those of direct susceptibility and differential vital staining.

1. Both basic and acid dyes were used, but with few exceptions basic dyes alone were found to be sufficiently penetrating and toxic in nearly neutral solutions to be effective.

2. Neutral salts and H ions in the medium retard visible staining with basic stains; OH ions and higher temperature facilitate it, as do also local injury and local healing.

3. Data are given, especially for methylene blue, demonstrating more or less satisfactorily: a staining gradient, sometimes a decolorization gradient, and always a disintegration gradient in the forms used, including *Paramecium, Dileptus gigas, Hydra oligactis*, several flatworms and oligochetes; and a staining gradient for the chick embryo. The loci of highest general direct susceptibility to most agents and conditions are first to stain visibly with the dyes, first to decolorize (in case color is lost) as death approaches, and first to disintegrate at death.

4. The disintegration gradient with basic dyes resembles that obtained with acids; disintegration with lethal acid dyes resembles the type obtained with alkalies and KNC (p. 361).

5. Basic dye color ions are positively charged, and evidently are taken up by negatively charged colloids and other anions, which there is

reason to believe are most numerous in metabolically active regions, where acid substances are produced (pp. 378–380).

6. There is much collateral evidence to the effect that a difference in permeability accompanies states and sites of greater metabolic activity; but there appears to be little justification as yet for distinguishing sharply between combining capacity and penetration power, or to attempt to give priority to either.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 57 OCTOBER 1, 1921 No. 3

EFFECTS OF PILOCARPINE UPON SALIVARY SECRETION IN NORMAL AND FEBRILE DOGS

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Received for publication June 13, 1921

Among the questions pertaining to the behavior of water in the body during fever is that of the changes in function in the digestive glands. In this connection Meyer, Cohen and Carlson (1) showed that the total quantity of the gastric secretion in human febrile cases is reduced.

The present investigations upon dogs were undertaken to determine whether the curve of salivary secretion is also affected by fever, and if so in what way. As saliva is not rapidly secreted by a resting animal several hours after food, the procedure adopted was to determine the response to a standard dose of pilocarpine in normal animals, repeating the procedure during fever after sufficient controls had been obtained.

METHODS. The following method of collecting saliva, similar to that used by Pawlow, Cushny (2) and others was employed:

Under ether and stringent asepsis a permanent salivary fistula was made in the right submaxillary (Wharton's) duct. This was done by ligating the duct as far forward as possible and then suturing it to the skin of the right submental region, after which it was incised. One month or longer was allowed for healing before experimentation.

One-half milligram pilocarpine hydrochlorid per kilo body weight of dog was selected as the standard dose. This amount was weighed

[1] The expenses of this work were defrayed from a grant from the Bache Fund of the National Academy of Sciences. The data are taken from a thesis presented by one of the authors (B.P.F.) in candidacy for the degree of Doctor of Medicine, Yale University, 1920.

and freshly made up in 8 to 10 cc. of distilled water for each individual experiment. Injections were made subcutaneously.

Throughout each experiment the animal was made to lie on a table on its left side, so that the salivary fistula was on the upper side. The fistula opening was then carefully wiped out and a small weighed pledget of absorbent cotton applied. The saliva secreted was collected by 5-minute periods upon such pledgets and the amount immediately ascertained by reweighing in order to prevent loss by evaporation. The secretion was thus observed for 10 minutes before and for 2 hours after the injection of pilocarpine.

Attention should be called to a number of the precautions which were taken. The dogs were disturbed as little as possible during the course of the observations and would lie quietly on the table, often even falling asleep. Furthermore the area surrounding the fistula opening was kept free of hair at all times so that nothing was weighed upon the tared cotton except the clean saliva. Besides this, great care was taken to prevent any fluid from the mouth reaching the field of operation and the saliva was blotted from the opening upon the cotton with the slightest and most uniform pressure possible.

Throughout the entire series of experiments the animals were kept on a standard diet consisting daily of canned meat, 8 ounces; bread, 4 ounces; lard, 2 ounces; water, as desired. The experiments were usually begun from 16 to 20 hours after the daily meal. The animals were allowed to run out of doors for several hours each day except in bad weather.

Two dogs were used. Dog 1 was a male tan mongrel terrier hound weighing 13 kgm. at the beginning of the pilocarpine experiments and 13.8 kgm. at the end (see fig. 3). At the time of operation, one month previous to the beginning of these experiments, he had weighed 12 kgm.

Dog 2 was a female black, short-haired mongrel weighing 7 kgm. at the beginning of the pilocarpine experiments and 6.7 kgm. at the end (fig. 4). At the time of operation, however, 2 months previously, she had weighed 8.25 kgm.

The pilocarpine injections were usually made at intervals of 2 or 3 days. One or two exceptions to this will be noted in figure 4.

The production of fever was accomplished by the subcutaneous injection of 1 cc. per kilo of a coli vaccine (Lederle) containing 1,000,000 million killed bacilli per cubic centimeter. After injection the temperature was taken at hourly intervals until it had risen consider-

ably. The determination of the salivary curve was then begun and the standard dose of pilocarpine injected.

RESULTS: *The effect of repeated injections of pilocarpine in normal dogs.* Contrary to expectation it was necessary to repeat the injection a number of times before a normal curve could be predicted within reasonable limits. In both dogs the maximum secretion for any 5-minute period was found to increase for a time with each successive injection. Furthermore the same was found to be true of the total saliva secreted within the standard period of 130 minutes. With respect to the total secretion, however, (see figs. 3 and 4) the increments in dog 2 were not very considerable after the first two injections.

These results are most clearly illustrated in figures 1 and 2, applying to dogs 1 and 2 respectively. The chronological order of the curves, which are superimposed, can be seen at a glance by comparing the respective maximum heights (the maximum usually occurred between the 25th and 40th minute of the experiment). In the case of dog 2 is seen a tendency for the curves to fall away more steeply from the maximum point; the greater the maximum secretion the steeper was the fall, thus accounting for the comparatively slight increments in the total secretion for each successive day (fig. 4). The failure of the totals to keep pace with the maxima is attributed in this case to the somewhat inferior nutritive condition of this dog.

From the above findings it is concluded that injections of pilocarpine repeated at intervals of a few days continue to hypersensitize dogs to the stimulating action of this drug upon the salivary secretion until the submaxillary gland is at least twice as responsive as after the first injection.

In order to determine whether this increasing hypersensitivity was due to a functional hypertrophy, the submaxillary glands on both sides of both dogs were removed at autopsy (subsequent to recovery from the fever experiments) in order to determine if possible whether anatomical hypertrophy was present. From neither the macroscopic findings nor from the size and character of the various types of cells as compared with those of normal dogs could any degree of anatomical hypertrophy be definitely established.

Effects of pilocarpine upon febrile dogs. The results obtained during fever in each animal differed in some respects and will be described separately.

Dog 1. On the 26th day after the first injection of pilocarpine had been made the animal weighed 13.9 kgm. and exhibited a temperature

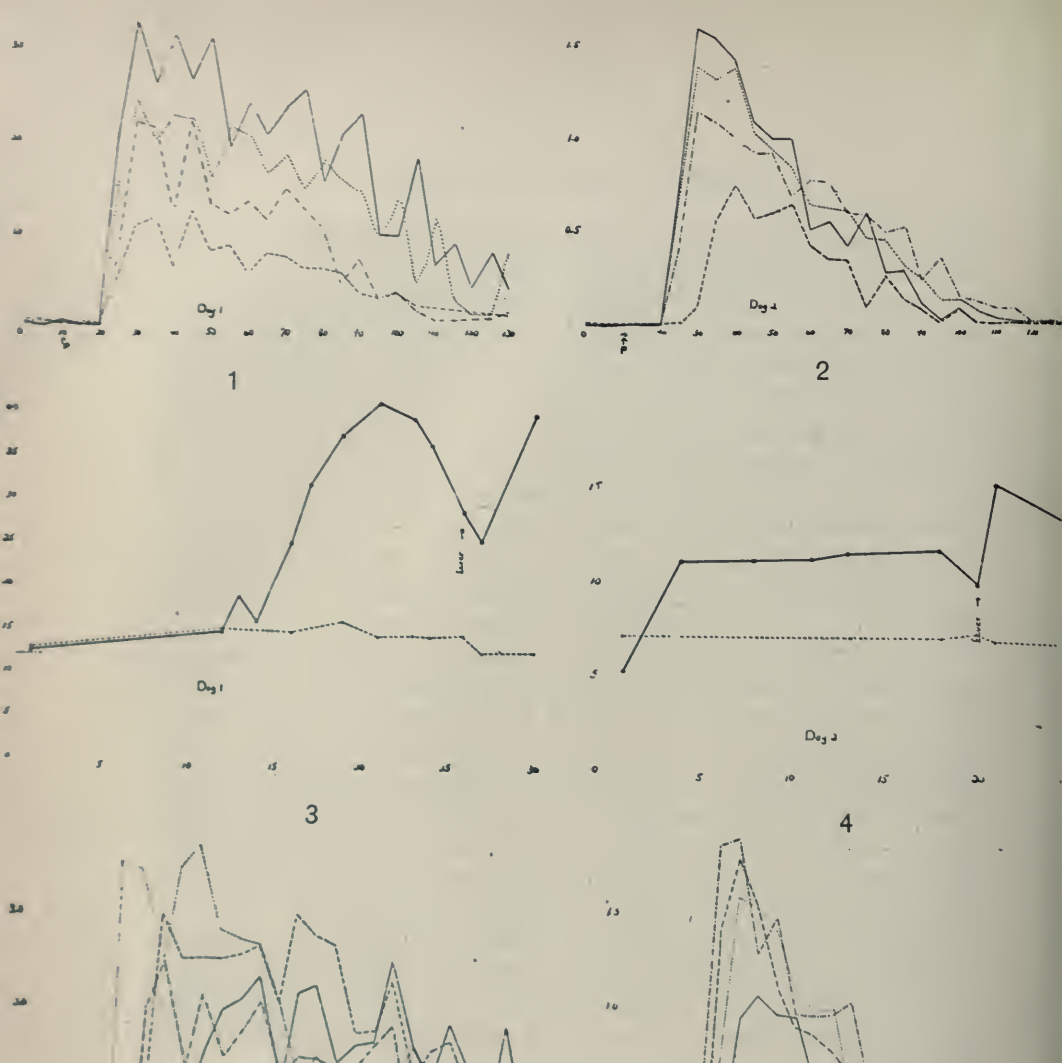

Fig. 1. Dog 1. Salivary secretion under pilocarpine 0.5 mgm. per kilo. Subcutaneous injection a
Curve of 1st day (----------); 13th day (-.-.-.-.-.); 17th day (.....); 21st day (————). Ordin
grams of saliva. Abscissae: minutes. (Note successively increasing maxima and total amounts of sal

Fig. 2. Dog. 2. Salivary secretion under pilocarpine 0.5 mgm. per kilo. Subcutaneous injection a
Curve of 1st day (----------); 4th day (-.-.-.-.-.); 13th day (.....); 18th day (————). Ordinates: g
of saliva. Abscissae: minutes. (Note successively increasing maxima. Total secretion did not inc
much after second injection (4th day).)

Fig. 3. Dog 1. Curves of total salivary secretion and of body weight on various days of pilocar
injections. Abscissae: days. Ordinates: grams saliva or kilograms body weight. Continuous line: t
secretion; broken line: body weight (the short broken line at the beginning indicates the weight at t
of operation). Day of coli injection indicated by arrow.

Fig. 4. Dog 2. Same as figure 3.

Fig. 5. Dog 1. Salivary secretion under pilocarpine 0.5 mgm. per kilo. Subcutaneous injection at
the normal animal and after induction of fever. Curve of 23d day (----------); 26th day (fever) (——
27th day (-.-.-.-.-.); 30th day (. . . . .).

Fig. 6. Dog 2. Salivary secretion under pilocarpine 0.5 mgm. per kilo. Subcutaneous injection at
the normal animal and after induction of fever. Curve of 18th day (. . . . .); 20th day (fever) (——

of 38.8°C. Fourteen cubic centimeters of coli suspension raised the temperature to 40.1°C. in 4 hours. Determinations for the curve of secretion were then begun and the pilocarpine was injected after the usual two preliminary 5 minute periods. The effect of the fever is best understood by a study of figures 3 and 5. Figure 3 shows that the total secretion fell from 35.7 grams on the 24th day to 28.2 grams on the fever (26th) day. There was a still further fall on the following (27th) day, although the temperature had returned to normal. In the meanwhile, however, the animal had refused his daily ration, the result of which is further seen in the weight curve (fig. 3, broken line). On the 30th day the animal although still low in weight had entirely recovered his power of response to pilocarpine, the total secretion for that day amounting to 38.8 grams.

In figure 5 the curve of secretion during fever is indicated by the continuous line, all of the other lines being broken. It will be seen that during the fever no marked secretion of saliva began until the 35th minute of the experiment. The maximum was not reached until the 95th minute, amounting to only 2.32 grams. This was lower than any maximum for this dog since the 14th day, before much hypersensitivity had been acquired. The completeness of recovery from the febrile condition is indicated by the curve of the 30th day (light dotted line) which presents two peaks, the first being reached at the 25th minute. Figure 5 should be compared with figure 1, in which other normal curves for this dog are presented.

Dog 2. On the 20th day after pilocarpine injections had been started in this dog, her weight was 7 kgm. and body temperature 38.1°C. To produce fever 7 cc. of the coli suspension were injected, the temperature rising to 39.8°C. by the end of the 2nd hour. The determinations for the curve of salivary secretion were then begun and pilocarpine injected as usual. The results obtained agree substantially with those on dog 1 during fever. The decrease in the height of the total secretion is well shown in figure 4, while the continuous line in figure 6 shows how low a maximum was reached. The maximum secretion of 1.09 gram (lower than any maximum observed since the first day of experimentation on this dog) was however attained with but comparatively slight delay, occurring at the end of the 30th minute.

On the following day the observations differed somewhat from those on the corresponding day in dog 1. Dog 2 exhibited on the day after coli injection some loss of weight due to the refusal of food, a slight

persistence of the febrile temperature, 38.8°C., being also observed. The curve of salivary secretion obtained at this time was found to be much higher than any of the pre-fever curves, the total amounting to 14.9 grams as against the greatest total of 11.5 grams on the 18th experimental day. During this copious secretion of saliva the temperature rose from 38.8° at the beginning to 39.7° at the end. On the succeeding days the curve of salivary secretion decreased until on the 27th day the pre-fever level was reached (11.1 grams).

Since in both dogs during the height of coli fever there occurred not only a definite delay in the appearance of secretory stimulation after pilocarpine, but also a marked reduction in the maximum secretion for any 5 minute period as well as in the total secretion, it must be concluded that the stimulation of salivary secretion by pilocarpine is less effective during fever.

The fever experiments were so conducted as to include the period when Barbour and Howard (3) have found constantly the greatest increase in the total solids of the blood as well as the highest temperature in coli fever. It seems therefore unnecessary to attribute, as others have done, the depression of secretion in fever to a somewhat indefinite condition of "cloudy swelling" of the glands. The paucity of secretion can be perfectly well accounted for by the lack of available water in the circulation (4).

The difference between the two dogs in the effects of pilocarpine on the day immediately after coli injection cannot at this time be accounted for. The one in which the fever still persisted (dog 2) exhibited a very marked stimulation of secretion, while the one in which the temperature had returned to normal (dog 1) secreted even less saliva than on the day of coli injection. Unfortunately no data are available as to the presence or absence of hydremia on these occasions. Undoubtedly both dogs were still in an abnormal condition; this is evidenced by the return to the pre-fever level of salivary secretion a number of days later, when the animals appeared to have recovered except for the local effects of the injection.

The marked increase of temperature observed in dog 2 following the pilocarpine injection on the day after coli injection can be accounted for readily by the copious loss of fluid from an animal already febrile.

Changes in the solid constituents of the saliva: In the second dog it was attempted to determine whether any quantitative changes occurred in the solid constituents of the saliva as the result of the

injections on the various days. To this end estimations were made of the total solids as well as of the total inorganic salts. The sample of saliva used in each case for this purpose was one of those taken near or at the highest peak of the secretion curve. It was collected in a weighed crucible held against the fistula opening. After weighing to determine the percentage of total solids a combustion was performed on the dried saliva and the total inorganic salts determined. Table 1 shows in the second and third columns the percentage changes in the solids and inorganic salts of the sample of saliva tested. Assuming the saliva to be of constant composition throughout the experiment we have calculated from the total saliva in 130 minutes (column 4) the total number of milligrams respectively of solids and salts probably secreted in that time (columns 5 and 6).

TABLE 1

Analysis of saliva (dog 2)

DAY OF EXPERIMENT	TOTAL SOLIDS	INORGANIC SALTS	SALIVA IN 130 MINUTES	ESTIMATED TOTALS IN 130 MINUTES	
				Solids	Inorganic salts
	per cent	*per cent*	*grams*	*mgm.*	*mgm.*
1	0.24		5.26	12.6	
4	0.79	0.29	11.01	87.0	31.9
8	1.11	0.21	11.01	122.2	23.1
11	1.00	0.56	11.07	110.7	62.0
13	1.08	0.42	11.40	123.1	47.9
18	1.27	0.71	11.50	146.1	81.7
20F	1.33	0.53	9.82	130.6	52.0
21	0.94	0.40	14.94	140.4	59.8

F = Day of coli injection.

As regards the total solids it will be seen that the saliva became more concentrated with each successive experiment of the first three in the normal series. Following this a comparatively constant composition was maintained until the coli injection, on which day a slight concentration is noted. However, the total volume of saliva was so much reduced on this day that the estimated total solids for the 130-minute period were considerably lower than during the experiment just previous. During the copious secretion of the day following fever the saliva was much more diluted.

The inorganic salts exhibit roughly a parallelism with the total solids except that they are distinctly diminished on the 1st and 2nd days of fever.

The data relative to the solid constituents are presented as a matter of record rather than a basis for final conclusions regarding changes in the composition of the saliva.

CONCLUSIONS

1. Salivary fistula dogs exhibit a gradually increasing sensitivity to pilocarpine during a series of repeated injections at intervals of 2 or 3 days. In two dogs the secretion increased with each experiment until both the total for 2 hours and the figure for the maximum individual 5-minute period were more than doubled.

2. This gradually increasing sensitivity to pilocarpine has not yet been explained.

3. Salivary response to pilocarpine becomes diminished during the height of coli fever in dogs. This diminution occurs under conditions in which an abnormally high concentration of the blood is known to obtain.

4. The depression of secretions during fever should probably be attributed rather to lack of available water than to "cloudy swelling" of the secretory glands.

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THE RÔLE OF TISSUE FIBRINOGEN (THROMBOKINASE) IN FIBRIN FORMATION AND NORMAL CLOTTING[1]

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Received for publication June 15, 1921

According to Wooldridge (1), tissue fibrinogen forms part of the fibrin it produces by action on the blood. This view has not been generally accepted owing, in part at any rate, to a general neglect of Wooldridge's work since that work and the ideas back of it were not in consonance with the current view of Schmidt (2) that clotting is due primarily to fibrin ferment or thrombin set free from the leucocytes on their disintegration. Morawitz (3) gave to tissue fibrinogen the name of thrombokinase to indicate his belief that it was an activator of thrombin. Fuld and Spiro (4) called it cytozym, supposing that it was an enzyme supplied by the tissues or leucocytes which served to activate prothrombin. The most recent view is found in the careful work of Howell (5) and his pupils that it removes or neutralizes in some way the antithrombin which is present in circulating blood. That these later conceptions are probably incorrect, or rather perhaps incomplete, and that Wooldridge was correct, is indicated by what follows.

That tissue fibrinogen clots blood in some other manner than thrombin is supposed to do, is shown by its clotting both *in vivo* and *in vitro*, whereas thrombin clots only *in vitro* This fact clearly indicates the importance of tissue fibrinogen in blood clotting as it normally occurs, that is, as the blood escapes from the vessels into the tissues. Wooldridge found that the addition of tissue fibrinogen to plasma increased the yield of fibrin recovered therefrom, but he had not yet reached a clear picture of the nature of the process of clotting and was ignorant of the rôle of calcium. Schmidt pointed out that the addition to plasma of the substance called by him paraglobulin increased the fibrin yield and he believed from this that paraglobulin played an impor-

[1] This work was financially aided by the U. S. Public Health Service and the Metropolitan Life Insurance Company, New York.

tant part in clotting, but subsequent work on the clotting of fibrinogen solutions in the absence of paraglobulin has caused the general neglect of the fact found by Schmidt.

It has been shown by Mills (6) that the active tissue coagulant is a protein-phospholipin compound probably consisting of about thirteen phospholipin molecules united to one protein molecule. The protein part alone and the whole compound have the solubility of globulins and are negatively charged in neutral salt solution, where they exist probably as sodium salts. It will be shown in the present report that when tissue fibrinogen is added to citrated plasma and clotting is then induced by calcium addition, tissue fibrinogen enters apparently without change into the fibrin formed and that only a part of the phospholipin of the added coagulant can thereafter be extracted from the fibrin, thus showing that the tissue fibrinogen is chemically bound. Analytical data will be presented to show that the missing phospholipin is present in the fibrin in a non-extractable form. It will also be shown that calcium is present in the fibrin in amounts varying somewhat with the composition of the fibrin. From these facts a diagram is given of the possible structure of fibrin molecules.

1. Is the active coagulant a true tissue fibrinogen? A fibrinogen may be defined as any organic albuminous substance capable of entering directly into fibrin formation, and to be found in the fibrin produced. We will now present evidence that this tissue coagulant is a true fibrinogen according to the above definition.

In all the experiments which follow horse plasma was used. The blood was drawn directly into vessels containing sufficient potassium citrate to give a final concentration of 0.5 per cent, the corpuscles allowed to sediment in the ice box and the clear plasma used. Such plasma required 0.35 cc. of 1 per cent $CaCl_2$ solution for each 1 cc. of plasma in order to get the quickest possible clotting at 40°C. but we actually employed an equivalent amount of 5 per cent $CaCl_2$ (7 cc. per 100 cc. plasma) to lessen the volume added. Citrate was used in preparing the plasma in the hope that there would be no calcium precipitate in the fibrin except that of the fibrin molecule.

The clots were allowed to stand at 40°C. for about 30 minutes after gelation to insure a completion of the process, and were then cut up with a knife and poured on large filters to drain. After standing in the ice box and draining off practically all the serum, the fibrins were thoroughly macerated on a fine steel screen with a steel spatula. These finely shredded fibrins, after washing with 0.9 per cent NaCl until free

from soluble proteins, and with distilled water until the washings were free from NaCl, were dried at 100°C. to a constant weight, and kept for analysis.

All tissue extracts used were sedimented for at least 24 hours in the ice box at 2°C. before being used, so as to leave little chance for having present anything except dissolved substances or those in a very fine state of suspension. The tissue fibrinogen was prepared from calf lung except in a few instances which are noted.

Some of the lung extracts were made from dried lung and others from fresh lung tissue as noted. In making the extracts from the *dried* lung (dried at room temperature) the lung was powdered and extracted with 0.9 per cent NaCl solution. To get an extract saturated with the lung proteins not over 4 cc. of the salt solution should be used for each gram of dried tissue. This gives a solution containing usually about 1.8 per cent of protein. With the *fresh* tissue about 2 cc. of salt solution for each gram of tissue yields a saturated extract. Such an extract of fresh lung contains about 2 per cent of protein. The fresh tissue was hashed in a meat grinder, ground well with sand in a mortar, the salt solution added and, after standing 10 to 30 minutes at room temperature, the extract was drained off through several layers of cheesecloth. After 24 hours sedimentation in cylinders at 2° to 3°C. these extracts showed little tendency to form further deposits. They were opalescent.

The extracts actually used were generally approximately saturated, but being made in part from dried lung not always powdered to the same degree of fineness and in part from fresh lung, they contained varying amounts of protein. The amounts are specified in each case.

We have found that lung extracts contain roughly four times as much globulin as albumin, that is, a saturated lung extract containing 2 per cent protein would contain 0.4 per cent albumin and 1.6 per cent globulin. As was pointed out in an earlier paper, the entire globulin fraction appears to be a single globulin, tissue fibrinogen.

In the analytical work the following methods were used:

For phosphorus: Pemberton-Neumann.

For nitrogen: Arnold-Gunning Modification of the Kjeldahl.

For calcium: The dried fibrins were first mixed with fusion mixture and ignited gently until white. The mass was then dissolved in 10 per cent HCl and Lyman's method (7) for determining the calcium followed from that point.

For phospholipin: Prolonged extraction with boiling 80 per cent and 95 per cent alcohol and absolute ether.

In the following series of experiments about 700 cc. of plasma were used in each experiment; where the amount was different from this, the yield of fibrin has been computed by proportionality for 700 cc. plasma in table 1 to make comparison of yield of fibrin more easy.

Inasmuch as the experiments consist in the comparison of the amounts of fibrin recovered from various samples of plasma with and without the addition of lung extract, the question arose as to the variation in amount of fibrin recoverable from the same sample of citrated horse plasma after standing for varying times. On testing this out it was found that the amount of fibrin yielded by such plasma varied widely, i.e., from 4.937 grams to 1.6975 gram for 700 cc. plasma, depending on the length of time the plasma was left standing in the ice box before using. There occurred a slow sedimentation, probably of the blood platelets or A-fibrinogen of Wooldridge, but there also took place in this layer of sediment a slow fibrin formation. After two weeks' standing of plasma at 3°C. this layer in the bottom of the flask was typical fibrin, while the supernatant plasma showed a greatly diminished fibrin yield on clotting by calcium addition. It is probable that the fibrin formation was caused by the presence of a slight amount of ionized calcium, the citrate being less effective in binding the calcium than is oxalate. No such fibrin formation takes place in plasma containing 0.5 per cent oxalate.

Inasmuch as the amount of fibrin varied so widely depending on the age of the citrated plasma, it was necessary in the following experiments to compare always the yield of fibrin under different conditions in a single sample of plasma. In this manner the variations were not of importance in interpreting the results, since controls were run for each series of experiments, and each series represents experiments carried out simultaneously. That the method of fibrin determination is accurate to within 1 per cent is shown by series G.

In series A the plasma stood 3 days before using; in series B, 5 days; in series C, 4 days; in series D, 3 days; in series E and F, about 10 days; in series G, about 2 weeks; and in series H, 1 day.

The first experiments, series A, 1, 2 and 3, show the great increase in fibrin yield produced by adding varying quantities of the extract of fresh lung tissue. Thus in experiment 1, 700 cc. plasma yielded without addition 4.116 grams fibrin; in experiment 2, in which 60 cc. fresh lung extract had been added to 700 cc. plasma, 4.502 grams fibrin were obtained; in experiment 3, the addition of 700 cc. of the same lung extract caused an increase of yield of the fibrin to 6.769 grams, an increase of 64.6 per cent.

The addition then of tissue fibrinogen (lung extract) to the plasma yielded a much firmer clot and a great increase in the amount of fibrin, the increases in later experiments varying up to 152 per cent, depending on the freshness of the lung tissue used and the concentration of the active substance in the plasma.

SERIES A: *Experiment 1.* Seven hundred cubic centimeters plasma + 700 cc. 0.9 per cent NaCl + 49 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 40° C. Normal control for fibrin yield from plasma. Weight of washed fibrin obtained: 4.116 grams.

Phospholipin extractable from this fibrin.....2.38 per cent
Phosphorus content after phospholipin extraction.....0.127 per cent
Calcium content before phospholipin extraction .....0.126 per cent

Experiment 2. Seven hundred cubic centimeters plasma + 60 cc. lung extract (made from fresh lung and containing 1.7 per cent proteins) + 640 cc. 0.9 per cent NaCl + 49 cc. 5 per cent $CaCl_2$. Clotted in 10 seconds at 40°C.

Weight of washed fibrin obtained.....4.502 grams
Increase in fibrin yield above normal.....9.38 per cent
Phospholipin extractable from fibrin.....4.37 per cent
Phosphorus content after phospholipin extraction..... 0.154 per cent
Calcium content before phospholipin extraction..... 0.186 per cent

Experiment 3. Seven hundred cubic centimeters plasma + 700 cc. lung extract (same as used in experiment 2) + 49 cc. 5 per cent $CaCl_2$. Clotted in 10 seconds at 40°C.

Weight of washed fibrin obtained..... 6.769 grams
Increase in fibrin yield above normal..... 64.46 per cent
Phospholipin extractable from fibrin..... 12.48 per cent
Phosphorus content after phospholipin extraction..... 0.493 per cent
Calcium content before phospholipin extraction..... 0.121 per cent

In order to discover whether the power of the extract to increase fibrin yield was dependent on the amount of phospholipin-protein compound present, or whether the protein without the phospholipin had the same power, experiment 4 was tried In this experiment the dried lung tissue had been partially extracted with benzene before extraction with NaCl so as to remove some of the phospholipin. In this case 5.273 grams fibrin were obtained, an increase of only 28.11 per cent as compared with 64.46 per cent in experiment 3, but the protein solution added was less than half as concentrated. The amount of protein added, however, was five times that in experiment 2, but the yield was increased only $2\frac{1}{2}$ times.

Experiment 4. Seven hundred cubic centimeters plasma + 700 cc. lung extract (described below) + 49 cc. 5 per cent $CaCl_2$. Clotted in $6\frac{1}{2}$ minutes at 40°C.

Weight of washed fibrin obtained..... 5.273 grams
Increase in fibrin yield above normal.....28.11 per cent
Phospholipin extractable from fibrin..... 6.22 per cent

The lung extract used in experiment 4 was made from lung tissue from which the phospholipin had been partially exhausted as follows: Calf lung tissue, dried at room temperature and powdered, was treated with benzene at room temperature (200 cc. benzene for 100 grams dried lung) for about 2 minutes. It was then freed from benzene and extracted with 0.9 per cent NaCl and the extract sedimented. The extract after sedimentation contained 0.7 per cent proteins. Such brief benzene extraction removed about 4.8 per cent of phospholipins, so that, whereas the dried lung tissue formerly contained 22.82 per cent phospholipin, it now had only 18.00 per cent. It will be shown in a separate paper on anticoagulants that this 4.8 per cent phospholipin comes mostly from the tissue fibrinogen of the lung tissue, the phospholipin of the remainder of the lung coming off with much greater difficulty. Thus extraction with benzene at room temperature for days cannot reduce the phospholipin content of the dried lung below about 15 per cent.

Since this experiment showed that removal of a portion of the phospholipin from tissue fibrinogen markedly reduced its power of entering into fibrin formation, experiment 5 was tried in which the phospholipin was more thoroughly extracted from dried lung tissue by benzene. The yield of fibrin (exper. 5) was now reduced to only 4.314 grams or 4.81 per cent increase in place of 28.11 per cent above, although the amount of protein added was almost twice that in experiment 4.

Experiment 5. Seven hundred cubic centimeter plasma + 700 cc. lung extract made as indicated below + 49 cc. 5 per cent $CaCl_2$. Clotted in 30 minutes at 40°C.

Weight of washed fibrin obtained	4.314 grams
Increase in fibrin yield over normal	4.81 per cent
Phospholipin extractable from fibrin	3.14 per cent

The lung extract used was made from lung tissue extracted with benzene at room temperature for 4 days, with change of the benzene 2 or 3 times a day. Such extraction reduced the phospholipin content of the lung tissue from 22.82 per cent to 15.27 per cent. The saline extract used above contained 1.33 per cent protein.

Finally the dried lung was completely extracted with benzene as in experiment 5, and then the phospholipin protein compound remaining in the saline extract of the defatted lung was removed by careful precipitation with N/2 H_2SO_4, leaving the protein free from phospholipin in solution. This extract was then added and although it contained 0.7 per cent protein, approximately the same as experiment 4, it no longer caused coagulation of blood, but inhibited it (exper. 6). The

fibrin yield after several days standing was so small as to be negligible. The fibrinogen remained unaltered in the solution and could be clotted by addition of active lung extract.

Experiment 6. Three hundred cubic centimeters plasma + 300 cc. lung extract (described below) + 21 cc. 5 per cent $CaCl_2$.

Only a fine fibrin network present after several days' standing. It was possible to induce clotting by addition of active lung extract.

The lung extract used above was made by taking the saline extract of benzene extracted lung, such as was used in experiment 5, and adding N/2H_2SO_4 to give a calculated concentration af about N/500. A portion of the globulin content of the extract precipitated at this point. This precipitated material contained about 13 per cent phospholipin, instead of the 41.6 per cent normally present in a precipitate obtained in a similar manner from active lung extracts. The solution remaining after removal of this precipitate contained 0.7 per cent protein, about half of which was globulin containing less than 1 per cent phospholipin. This globulin represented the active anticoagulant, or the part of the tissue fibrogen from which the phospholipin was most completely removed by the benzene extraction.

This series of experiments demonstrates two points: First, whereas a relatively small amount of lung extract will produce a maximal acceleration of the clotting process (exper. 2), the increase in the fibrin yield over normal is far less than when a larger amount of lung extract is used (9.38 per cent fibrin increase in exper. 2, as contrasted with 64.46 per cent increase in exper. 3). Second, partial removal of the phospholipin from the tissue fibrinogen by benzene, which leaves the protein as soluble as before (the difference in concentration of the extracts being due to alteration in fineness of the pulverized material and to variation in length of time of extraction), causes the lung extract to be much less effective in increasing the fibrin yield of a plasma, and causes the clotting time to be lengthened beyond normal. If the tissue fibrinogen still containing phospholipin in these extracts be removed by acid precipitation, then the remaining solution is so strongly antithrombic as almost entirely to inhibit fibrin formation in the recalcified plasma. It is possible to fraction this antithrombin solution and get a product which greatly delays fibrin formation, only a small amount of the fibrinogen being changed to fibrin after several days' standing. The anticoagulant action of this protein is considered in a forthcoming paper.

It is interesting to compare the increase in weight of the fibrin obtained with the amount of tissue fibrinogen added. Thus in experiment 2, series A, the actual increase obtained was 0.386 gram. There were added 60 cc. lung extract containing 17 per cent protein or 1.02 gram

protein of which about 0.8 gram was tissue fibrinogen. Of the total fibrinogen added 0.386 gram reappeared in the fibrin or 47 per cent of that added. In experiment 3, series A, there were added roughly 12 times as much tissue fibrinogen or about 9.6 per cent grams. The increase in fibrin was 2.653 grams or roughly a sevenfold increase in the amount of tissue fibrinogen bound in the fibrin. Since this tissue extract was made by simple salt extract of fresh lung tissue it contained albumins, approximately 20 per cent of the proteins, as well as tissue fibrinogen, the latter comprising its entire globulin content. It is clear that a considerable proportion of the tissue fibrinogen went into the fibrin either by a chemical or physical union during the clotting.

A second series of experiments (series B) similar to those of series A was now tried and very similar results were obtained, but in experiment 3 the increase in fibrin yielded was even higher than before and amounted to 80.9 per cent.

SERIES B: *Experiment 1.* One thousand cubic centimeters plasma + 1000 cc. 0.9 per cent NaCl + 70 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 40°C. Normal control for fibrin yield.

Weight of fibrin obtained	5.348 grams
Phospholipin extractable from fibrin	0.57 per cent
Phosphorus content after phospholipin extraction	0.09 per cent
Calcium content before phospholipin extraction	0.125 per cent

Experiment 2. Fifteen hundred cubic centimeter plasma + 1500 cc. lung extract (made from fresh lung tissue and containing 2.0 per cent proteins) + 1350 cc. 0.9 per cent NaCl + 105 cc. 5 per cent $CaCl_2$. Clotted in 15 seconds at 40°C.

Weight of fibrin obtained	8.656 grams
Increase in fibrin yield over normal	7.9 per cent
Phospholipin extractable from fibrin	2.91 per cent
Phosphorus content after phospholipin extraction	0.161 per cent
Calcium content after phospholipin extraction	0.116 per cent

In this experiment the minimum amount of lung-extract capable of producing maximal acceleration of clotting was used, and an increase of 7.9 per cent in the fibrin yield obtained as against 9.38 per cent increase in experiment 2 of series A.

Experiment 3. Five hundred cubic centimeters plasma + 500 cc. lung extract (same as used in experiment 2 above) + 35 cc. 5 per cent $CaCl_2$. Clotted in 15 seconds at 40°C

Weight of fibrin obtained	4.8375 grams
Increase in fibrin yield over noral	80.9 per cent
Phospholipin extractable from fibrin	17.55 per cent
Phosphorus content after phospholipin extraction	0.383 per cent
Calcium content before phospholipin extraction	0.141 per cent

Although Mills has stated in a previous paper (6) that the albumins of tissue extracts are inactive as regards blood clotting, we thought it

best to insert another series of experiments to prove beyond doubt that these fibrin increases are not due to the albumin content of the extracts but only to the globulin content, i.e., tissue fibrinogen. The following series (series C) proves these points. None of the fibrins of this series have been analyzed as yet.

SERIES C: *Experiment 1.* Six hundred cubic centimeters plasma + 600 cc. 0.9 per cent NaCl + 42 cc. 5 per cent $CaCl_2$. Clotted in 2 minutes 50 seconds at 41°C.
Normal control for fibrin yield.
Weight of fibrin obtained.....2.2750 grams

Experiment 2. Three hundred cubic centimeters plasma + 300 cc. lung extract (made from fresh calf lung and containing 1.8 per cent coagulable protein) + 21 cc. 5 per cent $CaCl_2$. Clotted in 10 seconds at 37°C.
Weight of fibrin obtained.....2.3470 grams
Increase in fibrin yield over normal.....106.3 per cent

Experiment 3. Three hundred cubic centimeters plasma + 300 cc. pure coagulant (as described below) + 21 cc. 5 per cent $CaCl_2$. Clotted in 10 seconds at 35°C.
Weight of fibrin obtained.....2.9425 grams
Increase in fibrin yield over normal.....158.7 per cent

The pure coagulant solution contained 1.23 per cent coagulable protein and was made as follows: a portion of the same lung extract as was used in experiment 2 was diluted with an equal volume of distilled water and precipitated by adding N/2 H_2SO_4 in proper amount. The precipitate was collected and washed in distilled water by sedimenting and decanting until all soluble albumins were removed. The precipitate was then dissolved in 0.9 per cent NaCl solution with the aid of N/2 NaOH sufficient to give a calculated strength of N/500.

It is seen from the above data that this preparation retains its accelerating action on clotting and gives a still greater increase in the fibrin yield than did the original lung extract although the concentration of tissue fibrinogen in the two solutions probably differed by less than 0.2 per cent. Out of 3.69 grams of pure coagulant used in experiment 3, 1.805 gram, or bout 50 per cent, was bound in the fibrin, while in experiment 2 only 1.2095 grams out of 5.4 grams of protein of lung extract were so bound.

Experiment 4. Two hundred cubic centimeter plasma + 200 cc. albumins of lung extract (containing 0.464 per cent coag. protein) + 14 cc. 5 per cent $CaCl_2$. Clotted in 3 minutes at 37°C.
Weight of fibrin obtained.....0.6910 gram

The albumin solution used was made by removing the globulin from lung extract by half saturation with $(NH_4)_2SO_4$, then obtaining the albumins by complete saturation, redissolving the albumins in water and dialyzing till free from $(NH_4)_2SO_4$. Dialysis is necessary, as the amount of sulphate present retards clotting to a marked degree.

It is seen here that the albumins alone have practically no effect on the speed of clotting. The fibrin yield is even a little less than normal; thus the normal yield was 0.3791 gram per 100 cc. plasma, while the yield here was only 0.3455 gram per 100 cc. plasma.

This series of experiments demonstrates clearly that the tissue fibringen, and that alone, enters into the fibrin formation, the albumins being totally inactive in this respect.

Having thus shown that tissue fibrinogen enormously increases the amount of fibrin, the question arises as to how it does so. The first suggestion is, of course, that it is simply entangled in the clot mechanically. The particles of tissue fibrinogen must be quite large even for colloids. Thus the solution even after sedimentation is opalescent and on filtering quickly clogs a filter, even a rather coarse one. It cannot be filtered through a bougie. This clogging may not be mechanical. It is quite possible that union occurs between the paper or the bougie, both of which are electro-negative, and the calcium or some other polyvalent electro-positive part of the tissue fibrinogen helps hold the latter in the filter. But in spite of this possibility certainly the first suggestion occurring to one is that the union is purely mechanical with the fibrin. Were this the case it ought to be possible to dissolve it out readily from the clot with salt solution, since it is easily, quickly and completely removed from dried lung tissue in this way, and also the phospholipin ought to be as readily and completely removable as it is from the dried tissue fibrinogen. The fact that it cannot be washed out with salt solution from the fibrin, and that a portion of the phospholipin cannot be extracted even on prolonged boiling with alcohol, as will be shown in a moment, indicates pretty plainly that the tissue fibrinogen is not simply mechanically entangled but is firmly chemically bound in the fibrin.

However, before taking this up, we tried first some experiments with other inert colloidal dispersions which were certainly as coarse as tissue fibrinogen, to see if they would similarly increase the weight of fibrin. The first which we tried was starch solution 0.4 per cent. It will be observed, series D, experiment 2, that the addition of an equal quantity of 0.4 per cent boiled starch solution only increased the fibrin yield 3.66 per cent, as contrasted with a 28 to 80 per cent increase when an equal volume of tissue fibrinogen solution was added. As the starch paste is only 0.2 per cent as contrasted with 1.0 per cent for the tissue extract in experiment 3, a better comparison is made with experiment 2, series A. Here the concentration of tissue fibrinogen in the final mix-

ture was only about 0.05 per cent yet the increase in fibrin was over 9 per cent. While, therefore, a small part of the increase might be due to the impossibility of washing out the tissue fibrinogen, yet the experiment indicates that the greater part of the increase is due to something else than mechanical entanglement.

SERIES D: *Experiment 1.* Seven hundred cubic centimeters plasma + 700 cc. 0.9 per cent NaCl + 49 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 40°C.

Weight of fibrin obtained	4.146	grams
Phospholipin extractable from fibrin	3.00	per cent
Phosphorus content after phospholipin extraction	0.127	per cent
Calcium content after phospholipin extraction	0.130	per cent

Experiment 2. Four hundred cubic centimeters plasma + 400 cc. 0.4 per cent starch paste (well boiled) + 28 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 40°C.

Weight of fibrin obtained	2.456	grams
Increase in fibrin yield over normal	3.66	per cent

Eighty-seven thousandth gram of starch, out of the 1.6 grams added, was bound in the fibrin. This experiment was inserted in order to test the effectiveness of our washing of the fibrins. It is seen here that inert suspended particles are not held in the fibrin to any great extent, even where the particles are so large as these of starch.

Experiment 3. Seven hundred cubic centimeters plasma + 700 cc. lung extract (made from dried lung several weeks after drying, and containing 2.09 per cent proteins) + 49 cc. 5 per cent $CaCl_2$. Clotted in 20 seconds at 40°C.

Weight of fibrin obtained	6.1675	grams
Increase in fibrin yield over normal	48.8	per cent
Phospholipin extractable from fibrin	14.77	per cent
Phosphorus content after phospholipin extraction	0.206	per cent
Calcium content after phospholipin extraction	0.133	per cent

We next tried the effect of another tissue extract, namely liver extract. The results show that this extract also increases the yield but not more than lung extract although it contains nearly three times as much protein.

SERIES E: *Experiment 1.* Four hundred cubic centimeters plasma + 400 cc. 0.9 per cent NaCl + 28 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 40°C.

Normal control for fibrin yield.

Weight of fibrin obtained	1.4387	grams

No analysis of this fibrin.

Experiment 2. Four hundred cubic centimeters plasma + 400 cc. liver extract (made from fresh calf liver and containing 5.9 per cent proteins) + 28 cc. 5 per cent $CaCl_2$. Clotted in 3 minutes 45 seconds at 40°C.

Weight of fibrin obtained	2.4933	grams
Increase in fibrin yield over normal	73.2	per cent

Weight of liver proteins bound in fibrin: 1.0546 grams or 4.74 per cent of the total proteins of the liver extract.

Experiment 3. Three hundred cubic centimeters plasma + 300 cc. lung extract (made from fresh calf lung and containing 1.82 per cent proteins)+ 21 cc. 5 per cent $CaCl_2$. Clotted in 50 seconds at 40°C.

Weight of fibrin obtained ..... 2.720 grams
Increase in fibrin yield over normal ..... 152.1 per cent
Weight of lung proteins bound in fibrin: 1.641 grams or 30.1 per cent of the total proteins of the lung extract.

This series demonstrates the effects of liver and lung extract in increasing the fibrin yield from a given plasma. Liver extract has only a slight accelerating action on clotting as contrasted to the effect of lung extract. Although the liver extract was more than 3 times as rich in proteins as the lung extract, still it increased the fibrin yield less than half as much (73.2 per cent increase from liver extract, and 152.1 per cent from the lung extract). Thirty and one-tenth per cent of the proteins of lung extract were bound in the fibrin, as contrasted with 4.47 per cent of the proteins of the liver extract. So far as precipitation reactions and appearances go, the globulin fractions of the two extracts are exactly alike, but the phospholipins extractable from these two globulins are very different in nature. The phospholipin from the lung globulin is a reddish brown substance, very hygroscopic, and very readily emulsified in water. That from the liver globulin is more yellowish in nature, much less hygroscopic, and almost impossible to emulsify in water. The protein fractions of the two globulins are alike in that they both strongly inhibit blood clotting *in vitro* or *in vivo*, and may be made to accelerate normal clotting by being joined to lung cephalin.

The differences noted in this series in regard to the fibrin yields resulting from use of liver and lung extracts are probably due to a difference in the nature of the phospholipins. This would certainly indicate that a direct chemical union is occurring in the fibrin formation and that the phospholipin is intimately concerned in that union.

Still more evidence that the increase of yield is due to chemical union was obtained by studying the increase due to the addition to the plasma of a boiled egg albumin suspension, egg white solution and gelatin (series F and G). While a decided increase (39.5 per cent) was obtained with the boiled egg white it was only about half as great as with a corresponding lung extract of the same protein content, and the unboiled egg white gives only a small increase (4.9 per cent) as contrasted with 60 to 70 per cent in the tissue fibrinogen. Moreover the precipitated coagulated egg white may be bound chemically in the fibrin also since it too is electro-negative and is easily precipitated by calcium.

In the case of gelatin, none was held in the fibrin clot so firmly that it could not be readily washed out.

Now tissue fibrinogen is very readily soluble in salt solution, just as is gelatin, and were it simply entangled it ought to be as easily removed.

SERIES F: *Experiment 1.* Four hundred cubic centimeters plasma + 400 cc. 0.9 per cent NaCl + 28 cc. 5 per cent $CaCl_2$. Clotted in 3 minutes 50 seconds at 40°C.

Normal control for fibrin yield.

Weight of fibrin obtained.....1.5705 grams

Experiment 2. Three hundred cubic centimeters plasma + 300 cc. egg albumin suspension + 21 cc. 5 per cent $CaCl_2$. Clotted in 3 minutes 10 seconds at 40°C.

Weight of fibrin obtained.....1.6445 grams

Increase in fibrin yield over normal.....39.5 per cent

The egg albumin suspension used was made by diluting fresh egg white with 5 times its volume of distilled water, stirring well, filtering, and boiling without stirring. This gave a milky suspension of the egg albumin, showing no sediment on 24 hours' standing, and being non-filterable through filter paper. It contained 2.12 per cent protein.

Of the 6.36 grams of suspended protein present here at the time of clotting, 0.4666 gram, or 7.33 per cent, was bound in the fibrin. This may be contrasted with the experiments where lung extract was used, in which usually about 30 per cent of the proteins of the extract were bound in the fibrin.

SERIES G: *Experiment 1.* Two hundred cubic centimeters plasma + 200 cc. 0.9 per cent NaCl + 14 cc. 5 per cent $CaCl_2$. Clotted in 5 minutes at 40°C.

Normal control for fibrin yield.

Weight of fibrin obtained.....0.4895 gram

Experiment 2. Two hundred cubic centimeters plasma + 200cc. 0.9 per cent NaCl + 14 cc. 5 per cent $CaCl_2$. Clotted in 5 minutes at 40°C.

Normal control for fibrin yield.

Weight of fibrin obtained.....0.4849 gram

Experiment 3. One hundred cubic centimeters plasma + 100 cc. egg white solution (described below) + 7 cc. 5 per cent $CaCl_2$. Clotted in 4 minutes at 42°C.

Weight of fibrin obtained.....0.2555 gram

Increase in fibrin yield over normal.....4.9 per cent

The egg white solution was made by mixing fresh egg white with 4 volumes of distilled water and filtering. It contained 2.6 per cent protein. About 4.6 per cent of this protein was bound in the fibrin formed.

Experiment 4. Two hundred cubic centimeter plasma + 200 cc. 2 per cent gelatin solution + 14 cc. 5 per cent $CaCl_2$. Clotted in 5 minutes at 42°C.

Weight of fibrin obtained.....0.4885 gram

Apparently none of the gelatin was bound in the fibrin.

This series shows, first, that the method of washing and obtaining the fibrin is accurate to within 1 per cent (expers. 1 and 2); and second, that protein solutions of about the same concentration as the tissue extracts used have little or no influence on the fibrin yield from a given plasma. Thus the fibrin yield in experiment 4 fell between the two normal controls, while experiment 3 with the egg white solution showed 4.9 per cent more fibrin than the higher one of the two normals. We conclude that the addition of other soluble proteins, such as fresh egg white and gelatin, to the plasma does not noticeably increase the fibrin yield.

Addition of non-filterable suspensions of inert insoluble substances, such as boiled diluted egg white and starch paste, causes some increase in the fibrin yield probably due to difficulty of washing the particles out, but the percentage of the total added material that is held in the fibrin is very much less than the percentage of the proteins of lung extract similarly bound. Thus 5.43 per cent of the added starch paste was held in the fibrin; 7.33 per cent of the boiled diluted egg white; and 30 per cent of the proteins of lung extract.

If the tissue extract is relatively inactive in accelerating coagulation it gives also a corresponding smaller increase in fibrin. Liver extract had 4.47 per cent of its protein content bound in the fibrin, giving a 73.2 per cent increase in the fibrin yield with a slight acceleration in time, while the lung extract had 30 per cent of its proteins bound and gave an increase of 152.1 per cent in the fibrin yield with a tremendous acceleration in rate (series E).

These facts clearly indicate that the active coagulant of lung extract enters chemically into fibrin formation rather than that it is merely held mechanically. This being the case, the substance may truly be called a fibrinogen, and it was therefore rightly named "tissue fibrinogen" many years ago by Wooldridge.

2. Part of the phospholipin of the added tissue fibrinogen is bound in the fibrin in a non-extractable form. If the tissue fibrinogen were held in the fibrin only mechanically, it should be possible to extract its whole phospholipin content, since before union with blood fibrinogen the phospholipin may be removed from it very readily by lipoid solvents. It was found, however, that in every case only a part of the phospholipin calculated from the gain of weight as being present in the fibrin, could be extracted even on prolonged treatment with boiling 80 per cent and 95 per cent alcohol and absolute ether. The values for the "calculated phospholipin" percentage in the fibrins were obtained by taking 41.6

per cent of the increase in fibrin weight, adding to this the weight of phospholipin to be extracted from the normal fibrin, and dividing this weight of phospholipin by the total fibrin weight. It was shown in a previous paper (6) that the purified coagulant contains 41.6 per cent phospholipin.

The following experiments (series H) show the analyses of fibrinogen and of fibrin after varying proportions of tissue fibrinogen have been added.

SERIES H: *Experiment 1.* Several grams of blood fibrinogen were prepared for analysis by adding to citrated horse plasma an equal volume of saturated NaCl solution, redissolving the precipitate in distilled water and reprecipitating twice, washing in ½ saturated NaCl solution several times, and finally freeing from salt by coagulating by boiling and washing in distilled water.

This fibrinogen yielded 0.71 per cent phospholipin; after extraction it still contained 0.112 per cent P and 0.092 per cent Ca. Nitrogen content before extraction was 16.54 per cent, and afterwards was 16.62 per cent.

Experiment 2. Eight hundred cubic centimeters plasma + 800 cc. 0.9 per cent NaCl + 56 cc. 5 per cent $CaCl_2$. Clotted in 3 minutes at 42°C. (Control for fibrin yield.)

Weight of fibrin obtained	5.642 grams
Phospholipin extractable from fibrin	1.72 per cent
Phosphorus content after phospholipin extraction	0.127 per cent
Calcium content before phospholipin extraction	0.129 per cent
Nitrogen content before phospholipin extraction	15.74 per cent
Nitrogen content after phospholipin extraction	16.14 per cent

Experiment 3. Eight hundred cubic centimeters plasma + 800 cc. lung extract (2.06 per cent protein) + 56 cc. 5 per cent $CaCl_2$ Clotted in 23 seconds at 42°C.

Weight of fibrin obtained	7.652 grams
Increase in fibrin yield over normal	34.03 per cent
Phospholipin extractable from fibrin	7.50 per cent
Phosphorus content after phospholipin extraction	0.209 per cent
Calcium content before phospholipin extraction	0.120 per cent
Nitrogen content before phospholipin extraction	15.18 per cent
Nitrogen content after phospholipin extraction	15.76 per cent

The lung extract used in this experiment was made from dried calf lung that had stood in the laboratory for several weeks and so had lost considerable of its activity. Thus clotting occurred after 23 seconds as contrasted with 10 seconds in series A, and the fibrin yield was increased here only 34.03 per cent.

This series was carried out in order to get fibrinogen, normal fibrin, and fibrin containing tissue fibrinogen, all from the same sample of plasma, for analysis and comparison. The results of all the analyses will be found summarized in table 1.

TABLE 1

Results of fibrin analyses

SERIES	EXPERIMENT NUMBER	WEIGHT OF FIBRIN	WEIGHT OF FIBRIN INCREASE	FIBRIN INCREASE	CALCULATED PHOSPHOLIPIN	EXTRACTABLE PHOSPHOLIPIN	PHOSPHORUS		CALCIUM	
							Before phospholipin extraction	After phospholipin extraction	Before phospholipin extraction	After phospholipin extraction
		grams	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	1	4.116				2.38		0.127	0.126	
	2	4.502	0.386	9.38	5.95	4.37		0.154	0.186	
	3	6.769	2.653	64.46	18.69	12.48		0.493	0.121	
	4	5.273	1.157	28.11		6.22				
	5	4.314	0.198	4.81		3.14				
B	1	3.744				0.57		0.09	0.125	
	2	4.040	0.296	7.90	3.86	2.91		0.161		0.116
	3	6.773	3.029	80.90	19.17	17.55		0.383	0.141	
C	1	2.604								
	2	5.476	2.872	106.3						
	3	6.865	4.261	158.7						
	4	2.419								
D	1	4.146				3.00		0.127		0.130
	2	4.298	0.152	3.66						
	3	6.168	2.022	48.80	16.64	14.77		0.206		0.133
E	1	2.518								
	2	6.349	3.829	152.10						
	3	4.363	1.843	73.20						
F	1	2.748								
	2	3.837	1.089	39.50						
G	1	1.713								
	2	1.698					0.177			
	3	1.788	0.075	4. 90						
	4	1.710								
H	1					0.71		0.112		0.092
	2	4.937				1.72		0.127	0.129	
	3	6.617	1.680	34.03	12.28	7.50		0.209	0.120	

That the missing phospholipin is present in the fibrin in a firm union is evident from the following facts:

1. Nitrogen determinations (Arnold-Gunning modification of Kjeldahl method) before and after phospholipin extraction by boiling alcohol, ether, etc., gave these results in series H:

	NITROGEN BEFORE EXTRACTION	NITROGEN AFTER EXTRACTION
	per cent	*per cent*
Exper. 1. Fibrinogen	16.54	16.62
Exper. 2. Normal fibrin	15.74	16.14
Exper. 3. Fibrin with 34 per cent increase due to tissue fibrinogen	15.18	15.76

Hammarsten (8) gives 16.68 per cent N for pure fibrinogen free of lipoids, which agrees with our first determination. Normal fibrin apparently contains some non-protein material in non-extractable form, since its N is still below that of fibrinogen, while the fibrin containing considerable tissue fibrinogen shows still more non-protein material not extractable as lipoid. It has been shown previously (6) that tissue fibrinogen contains 10.7 per cent N before phospholipin extraction and 16.06 per cent N afterwards. If all the phospholipin of the added material were extractable, the nitrogen percentage of the fibrin should be between 16.06 and 16.62 per cent.

2. Phosphorus determinations (Pemberton-Neumann method) on fibrins after phospholipin extraction show the presence of more phosphorus in the fibrins containing the tissue fibrinogen than in normal fibrin. Thus, normal fibrin after alcohol and ether extraction still contains about 0.127 per cent P., which is a little higher than fibrinogen; while fibrins containing tissue fibrinogen show 0.209 per cent to 0.493 per cent P, depending on the amount of the fibrin increase. In experiment 3 of series A, calculation of the phosphorus in the added 64.96 per cent of tissue fibrinogen and deduction from this of the phosphorus in the 12.48 per cent phospholipin extracted, gives 0.371 per cent P in the fibrin, contributed by the added tissue material. If we add to this the fixed phosphorus content of the normal fibrin, 0.127 per cent, the result is 0.498 per cent P, which agrees with the actual finding of 0.493 per cent P.

It certainly seems evident, then, that the entire amount of non-extractable phospholipin can be accounted for by the phosphorus determinations. These findings also furnish evidence that it is the entire tissue fibrinogen molecule that enters into the fibrin formation and not

the phospholipin alone and that little else of the added tissue extract could possibly be concerned.

3. Further proof that there is some phospholipin in the fibrin in a non-extractable form is supplied by extraction of such fibrins following digestion by lipase-free trypsin solutions. Considerable additional phospholipin may be extracted following such digestion, but the experiment has not as yet been sufficiently controlled to supply exact data. The results will be given later.

The evidence appears to the writers to be conclusive that in the union of tissue fibrinogen to blood fibrinogen, a certain amount of the phospholipin of the tissue fibrinogen is bound in the fibrin in a non-extractable form. It is difficult to see how this could be brought about unless the phospholipin had taken on an additional point of attachment to hold it more firmly in the fibrin molecule, this new attachment probably being to the blood fibrinogen, directly or indirectly. This impossibility of extraction of the phospholipins even by boiling alcohol is the more striking when it is remembered that the phospholipin may be extracted from the tissue fibrinogen alone with great ease and even by the use of benzene at room temperature.

3. How are tissue and blood fibrinogen united in fibrin formation? Calcium percentages. It being apparent that a chemical union does take place between tissue and blood fibrinogen during clotting—and only then—the question arises as to the nature of this union. Again certain facts must be borne in mind:

1. Both tissue and blood fibrinogen are electro-negative in neutral solution, as is readily shown by cataphoresis experiments.

2. Calcium is necessary for the clotting process as here carried out, and enters into the fibrin in definite amounts. (It will be noted that these analyses do not deal with fibrin formed from the action of purified thrombin on purified fibrinogen. Free calcium is apparently not necessary for such clotting, the Ca already present probably being sufficient.)

3. In the union of the two fibrinogens a part of the phospholipin of the tissue fibrinogen is firmly bound in the fibrin in a non-extractable form.

4. Removal of the phospholipin from the tissue fibrinogen in a manner to leave the protein part soluble destroys its thromboplastic power and instead it becomes strongly anticoagulant in action, and prevents the union of the tissue substance with blood fibrinogen in fibrin formation (series A, exper. 6).

From the above stated facts, the authors infer that the fibrin molecule is probably formed by a union of the tissue and blood fibrinogens, the union possibly being between the phospholipin of the tissue fibrinogen and an acid group of the blood fibrinogen.

This union of the two fibrinogens is probably brought about by the replacement of the H^+ ion (or Na^+ ion if they exist as salts) of the phospholipin and of the blood fibrinogen by a Ca^{++} ion, the resulting complex calcium salt being insoluble and possessing the property of gelation. That the process is a calcium salt formation is strongly indicated by an experiment suggested to us by Dr. G. H. A. Clowes in December, 1920. That is, whereas 1 cc. of citrated plasma + 1 drop of lung extract will clot in a certain time at 40°C. (for instance, 40 seconds) after adding $CaCl_2$, no gelation will occur if sufficient oxalate be added to bind the free calcium as late as only 5 seconds before the expected time of gelation, only a weak, stringy clot forming on standing 24 hours. However, if this mixture, at 40°C., be recalcified, clotting occurs in the usual fashion, except that only 10 to 15 seconds are required now, instead of 40 seconds. This indicates that the gelation phase of clotting represents the condition where sufficient fibrin molecules have formed to precipitate from solution to form a gel, and that the union of the two fibrinogens may be stopped at any point by removing the free calcium ions from the solution. This experiment is rather difficult to explain by the thrombin theory of fibrin formation.

It is impractical to review here all the literature that has appeared relative to the action of calcium in blood clotting since the work by Greene (9) and Arthus and Pages (10) demonstrated its importance in the process. It has been considered by most investigators that calcium is necessary for the production of active thrombin, but the question as to whether thrombin itself contains calcium, by virtue of which it is able to clot fibrinogen solutions, has never been satisfactorily answered. Most of the work done in this direction has been done with so-called "purified" substances (fibrinogen, prothrombin, thrombin) which are very likely greatly altered in their solubility and chemical reactivity. It was partly for this reason that we attacked the problem through the use of citrated plasma and tissue extracts without preliminary purification of the reactive factors concerned in the clotting. We believe that we are thus working with conditions more nearly simulating those present in the normal clotting of blood escaping from a ruptured vessel over injured tissues.

We must refer briefly to a recent paper by Mason (11) in which he reviews the literature of coagulation and arrives at two conclusions but

gives no experimental results in support of them. He suggests that both fibrinogen and thrombin are kept inactive by the presence in each of a "protective colloid" which must be removed before clotting can take place. After these protective proteins are removed the "precipitating" portions of thrombin and fibrinogen are joined through calcium. This idea, while somewhat like ours reported in December before his paper appeared, is in other respects very different. The idea of colloids being thus precipitated by polyvalent metals was pointed out by A. P. Mathews (12) in 1905, when he suggested that the ability of such metals to precipitate colloids was probably due to their multivalence making it possible to unite two or three or more colloidal aggregates into very large aggregates, the union being through the polyvalent metal. Thus trivalent metals are better precipitating agents for negatively charged colloids than bivalent ones of the same atomic weight while monovalent metals possess no precipitating power except in high concentration. We are restricted from using trivalent metals for fibrin formation as we would like by the very active precipitating power they possess, all the proteins present in plasma being precipitated in a mass. With the divalent metals (Ca, Ba, Sr, Mg), however, the more easily precipitable proteins of plasma are acted upon first and we get normal fibrin formation. Now fibrinogen is the least soluble of the plasma proteins. It separates out first in almost every method of protein precipitation, such for instance, as salting out and acid precipitation or by heat coagulation. The same is also true of the tissue fibrinogen of tissue extracts. Therefore it is not surprising that these two colloids should be the ones to precipitate by union through calcium. Casein apparently enters into the clot in the same manner when present in plasma at the time of clotting. And the clotting of paracasein is no doubt entirely similar to blood clotting in this respect.

As for the presence of "protective colloids" in blood fibrinogen, prothrombin and the active principle of tissue extracts, Mason gives no proof of this except by referring to the work of Hammersten, Schmiedeberg and Heubner on the presence of a soluble globulin left in the serum following the clotting of fibrinogen solutions. This was formerly taken as proof that the fibrinogen was hydrolyzed in the process into a soluble and an insoluble fraction, but few men at present believe this to be likely. The work of Rettger (13) on the quantitative reaction between thrombin and fibrinogen at once raises the question whether this soluble serum globulin might not have been merely the excess of

fibrinogen left after the thrombin was exhausted. Some more definite proof of the presence of such "protective proteins" is necessary before such a theory of clotting can be seriously considered, although the idea of there being such a possibility is to be commended.

To return again to the manner of union of the two fibrinogens, it is known that phospholipins are both acids and bases, that is, they are amphoteric, like proteins. The union of the phospholipin and protein in tissue fibrinogen might then be either through the acid group of the former and amino group of the latter, or vice versa. However, in order to have an ionizable H^+ or Na^+ ion on each phospholipin, it is necessary to assume that the amino of the phospholipin attaches to the protein. It is only thus that we could account for this phospholipin joining to blood fibrinogen through calcium. Attempts to prove this linkage are in progress.

Taking the molecular weight of cephalin as about 890, and that of protein as 16,000, the tissue fibrinogen with its 41.6 per cent phospholipin would consist of 13 molecules of the phospholipin attached to one protein molecule, giving a molecular weight of 27,400 (fig. 1). A solution of such molecules, as lung extract, is not filterable even through filter paper after the first few cubic centimeters have passed and clogged the filter pores.

It has been observed that a relatively small amount of lung extract will give a maximum acceleration of clotting; for instance, one drop of a saturated lung extract in almost every case quickens the clotting of 1 cc. of plasma just as much as does 1 cc. of the extract. However, the increase in weight of the fibrin is quite different in the two conditions, often being 16 to 18 times as great in the latter condition. We might suppose that the minimum amount produces maximum acceleration when a point of attachment to the tissue fibrinogen has been given to each blood fibrinogen molecule. That would mean that one blood fibrinogen molecule would be attached to each of the 13 phospholipin molecules of the tissue fibrinogen, giving a molecular weight to the complex of about 235,900 (fig. 2). About 11.6 per cent of such fibrin would be tissue fibrinogen, while we found that the addition of sufficient tissue fibrinogen to give 9.38 per cent increase in fibrin weight actually did produce such a maximum acceleration of clotting. Such fibrin, according to our idea, should contain 13 atoms of calcium or 0.216 per cent, and 0.177 per cent P. Actual determination gave 0.186 per cent Ca and 0.154 per cent P (series A, exper. 2).

Our theoretical maximum fibrin increase should represent one tissue fibrinogen molecule united to one blood fibrinogen molecule (fig. 3). The molecular weight would be about 43,400, with the added tissue

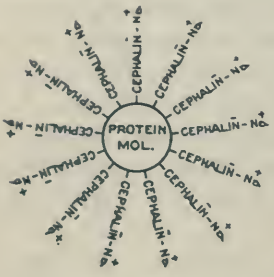

FIG. 1

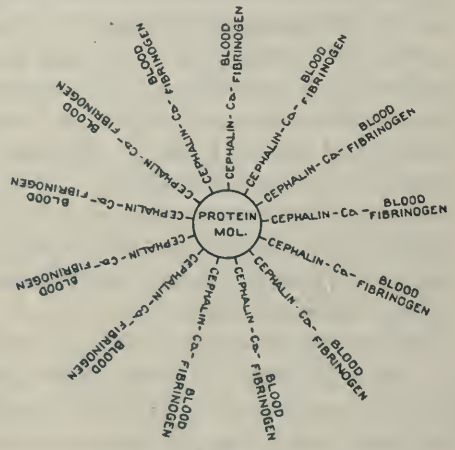

FIG. 2

FIG. 3

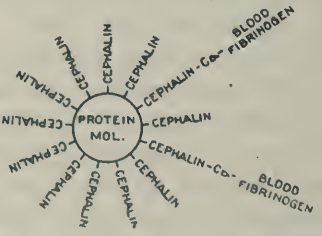

FIG. 4

Fig. 1. Tissue fibrinogen molecule (1 protein molecule to 13 phospholipin molecules. Mol. wt. 27,400.)

Fig. 2. Fibrin molecule obtained by adding minimum amount of tissue fibrinogen to produce maximal acceleration of the clotting. (Mol. wt. 235,900.)

Fig. 3. Fibrin molecule representing a maximal fibrin increase resulting from tissue fibrinogen addition. (Mol. wt. 43,400.)

Fig. 4. Fibrin molecule representing two blood fibrinogens joined to one tissue fibrinogen. (Mol. wt. 59,400.)

fibrinogen being 171 per cent as much as the blood fibrinogen. In our experiments so far, the highest fibrin increase we have been able to obtain is 152 per cent (series E, exper. 2). In series B, experiment 3, an increase of 80.9 per cent in the fibrin yield was obtained and the

fibrin analyzed. This increase represents about one tissue fibrinogen molecule to two blood fibrinogens, and should, therefore, have a molecular weight of about 59,400 (fig. 4). It should contain two calcium atoms, or 0.134 per cent Ca before phospholipin extraction and 0.369 per cent P after phospholipin extraction, assuming that all the phospholipin can be removed except that between the two protein molecules. Actual determinations gave 0.141 per cent Ca and 0.383 per cent P under these conditions. This fibrin should have $\frac{1}{13}$ of its phospholipin in extractable form, which would give 16.13 per cent phospholipin from the fibrin. The actual yield was 17.55 per cent.

On the whole, the fibrin analyses gave results checking closely enough with our idea of the structure of the fibrin molecules to warrant us in concluding that our idea is a possible one. We feel, then, that it is probable from the facts adduced that in the clotting of plasma containing dissolved tissue-fibrinogen, fibrin formation is a direct chemical union of the tissue and blood fibrinogen. The evidence leads us to believe that this union is between the blood fibrinogen and the phospholipin of tissue fibrinogen with calcium as the connecting link between the two. We have shown that clotting is almost impossible in the absence of this phospholipin fraction, just as it is impossible in the absence of calcium or other bivalent metals.

We may infer that this same kind of fibrin formation occurs in all cases of hemorrhage where the escaping blood flows over injured tissues. In fact this might well be called the "normal" method of fibrin formation, in the sense that it is the physiological method developed by the organism to prevent undue loss of blood from the vascular system. Two very easily precipitable colloids, one in the blood and the other in the body tissues, form almost an instantaneous union through calcium when they are mixed by escape of the blood over the tissues.

As regards the method of fibrin formation in plasma containing no tissue extract, a condition which may be called the abnormal method, although it has generally been studied as the normal one, our results are very interesting, but we wish to withhold discussion of them for the present. Some further proof is required before we can fully formulate our ideas of this process.

SUMMARY OF RESULTS AND CONCLUSIONS

1. The fibrin yield from a given plasma may be made to vary greatly by tissue extract addition, variations being noted up to 152 per cent above normal.

2. The globulin fraction of the tissue extract is found to be capable of thus increasing the fibrin yield. Therefore we may conclude that this globulin is a true tissue fibrinogen.

3. The albumins of tissue extracts, and other soluble proteins such as egg white and gelatin, are inactive as regards blood clotting when added to plasma and do not increase the fibrin yield.

4. Removal of the phospholipin from the tissue fibrinogen by the use of benzene at room temperature, lessens or destroys the ability of the globulin to enter into fibrin formation, the loss of coagulative power depending on the completeness of the phospholipin extraction.

5. Thorough phospholipin extraction (boiling 80 per cent and 95 per cent alcohol and absolute ether) of fibrins containing added tissue fibrinogen shows that not all the phospholipin calculated as being present can be extracted. Phosphorus analyses show that these fibrins, even after thorough phospholipin extraction, contain 0.154 per cent to 0.493 per cent P as compared to 0.127 per cent P in normal fibrin.

6. The nitrogen content of fibrin containing added tissue fibrinogen is found to be below that of normal fibrin, the difference depending on the amount of added tissue material. After removing all extractable phospholipin, the difference is less, but is still quite definite, so that we may conclude that some non-protein material is present in the fibrin in a non-extractable form. Considering the results of the phosphorus analyses, it is very probable that this material is a part of the phospholipin of the added tissue fibrinogen.

7. Calcium is found in the fibrins in amounts varying somewhat with the composition of the fibrin. These calcium percentages are roughly such as would be found if the union of the blood and tissue fibrinogens was through a calcium atom in each case. This, taken together with the fixation of definite amounts of phospholipin in the fibrin, makes it probable that the union of the two fibrinogens is between the phospholipin of the tissue fibrinogen and an acid group of the blood fibrinogen, calcium being the connecting link. Diagrams are given illustrating the probable composition of tissue and blood fibrinogen and fibrin.

8. The composition of fibrin may be made to vary quite widely by varying the amount of tissue fibrinogen present at the time of clotting.

9. Tissue fibrinogen (thromboplastin) probably does not then produce coagulation of the blood normally by removing an antithrombin or anticoagulant, as has been suggested by Howell, or by acting as a thrombokinase, but it itself unites directly, through calcium, with blood

fibrinogen to make fibrin. On the other hand, if antithrombin is present tissue fibrinogen will neutralize its action, as Howell suggested.

10. This explanation clears up the rôle in clotting of both calcium and tissue fibrinogen. The significance of thrombin will be considered later.

Note: The authors wish to express their appreciation of the helpful criticism of Dr. A. P. Mathews in the preparation of the manuscript, and their gratitude for his suggestions in the course of the work.

Note: The major part of the work here reported was carried out in the summer of 1920, and was reported in brief before the Joint Session of the Federation of American Societies for Experimental Biology, meeting in Chicago, December 28, 1920. The abstract of this report appeared in the Journal of Biological Chemistry, volume xlvi, page viii of the Proceedings, in the March number.

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A CONTRIBUTION TO THE STUDY OF THE RELATION BETWEEN VITAMIN-B AND THE NUTRITION OF THE DOG[1]

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Received for publication June 15, 1921

The essential facts known concerning the significance of vitamin-B in the nutrition of animals have been ascertained in studies chiefly on pigeons, rats and mice. The relation of this vitamin to the nutrition of the dog has been studied only very recently.. Voegtlin and Lake (1) and Karr (2) demonstrated that in the dog, as in the other species which have been studied, symptoms resembling polyneuritis will develop if the animal is fed for a considerable period on a diet which lacks vitamin-B.

Voegtlin and Lake emphasized loss in body weight as one of the symptoms shown by a polyneuritic dog. Karr, however, attributed this to a loss of appetite, and succeeded in showing some relation to exist between the desire to partake of food and the administration, by means of a stomach sound, of brewery yeast, or bakers' yeast, or a suspension made by squeezing canned tomatoes through cheesecloth. He concluded that the restoration of appetite, which resulted from the administration of any one of these products, was due to the vitamin-B which it contained, since, in the case of yeast, the product was less potent in this respect after it had been autoclaved.

Voegtlin and Lake used autoclaved lean meat to produce symptoms resembling polyneuritis in dogs and cats. Karr, on the other hand, prepared a series of dietaries consisting of casein or wheat gluten as the sole source of protein, and sucrose and lard to supply the remaining calorific requirements. The contention of the first-named authors that "the dietary habits of the various animals had to be taken into consideration"

[1] The data in this paper are taken from a dissertation presented to the faculty of the Graduate School in Yale University, 1921, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

does not seem to be a very important one in view of the success which attended Karr in his use of a "synthetic" dietary with the dog.

The data presented in this paper and constituting an amplification of that reported by Karr were obtained by following essentially the methods employed by him.

EXPERIMENTAL PART: *Foods.* What is designated diet I in this paper is identical with Karr's diet I. Diet II, as prepared by the writer consisted of a slight modification of diet I, the alteration being simply the substitution of butter fat for an equivalent amount of lard in a quantity sufficient to constitute about 7 per cent of the food. The desire to perfect the dietary in all respects and to make it as far as possible deficient in only one factor, namely, vitamin-B, was the chief reason for supplying butter fat as a source of vitamin-A.

Although Karr obtained no evidence indicating specifically that the absence of vitamin-A resulted in injury to the experimental animals within the period of his tests, it does not follow that dogs fed on his diet should show precisely the same syndrome, when pathological symptoms do appear, as do dogs which are fed on a diet which includes vitamin-A. Another improvement of the diet would be the addition to it of the antiscorbutic vitamin. To do this without at the same time adding vitamin-B, however, does not appear feasible at the present time.

Diet I (from Karr (2)

	grams
Commercial casein (12.7 per cent N)	6.3
Sucrose	4.5
Lard	4.1
Bone ash	0.4
*Salt mixture	0.2
Water	2.5
	18.0

Containing 0.8 gram N. and 80 calories

Diet II

	grams
Commercial casein (12.7 per cent N)	6.3
Sucrose	4.5
Butter fat	1.1
Lard	2.8
Bone ash	0.4
*Salt mixture	0.2
Water	2.7
	18.0

Containing 0.8 gram N. and 80 calories.

* Salt mixture (from Karr (2))	grams
Sodium chloride	10
Calcium lactate	4
Magnesium citrate	4
Ferric citrate	1
I-KI solution	a few drops

Sources of vitamin-B. The following products containing vitamin-B were used in the experiments reported in this paper.

Dried brewery yeast: administered in the form of a suspension in water.

Neutralized tomato juice: from canned tomatoes of commerce. The tomatoes were squeezed through cheese cloth and the suspension centrifuged. The supernatant fluid was heated to 80°C., carefully neutralized with sodium hydroxide, and filtered through paper. The filtrate was administered by stomach sound.

Alcoholic extracts of wheat embryo, rice polishings and navy bean: The method of McCollum and Simmonds (3) was used in preparing these extracts. The raw material was first extracted with ether. It was then extracted repeatedly with hot ethyl alcohol, the alcoholic fractions combined, the bulk of the alcohol removed by distillation under diminished pressure at temperatures ranging between 40° and 50°C., and the concentrated alcoholic solution evaporated to dryness in shallow porcelain dishes. The yellow gummy residue was taken up in distilled water and the aqueous solution filtered through paper. This solution was tested with litmus and carefully neutralized with sodium hydroxide. It was then made up to such a volume that 1 cc. of the final solution represented the extract from either 1 or 2 grams of ether-extracted material.

The neutralized tomato juice and the extracts of wheat embryo, rice polishings and navy bean, were tested for vitamin-B upon polyneuritic pigeons with positive results.

Influence of vitamin-B on food intake. At the beginning no difficulty was experienced in making the dogs eat the food offered. As Karr has already pointed out, however, the animals, after having been fed on the vitamin-free food for a short time, differed as to the amount of the food which they would eat daily. All of them ate these diets for a varying period. A loss of appetite then revealed itself resulting in a lowered and irregular daily food intake and occasionally in a complete refusal of the food over many days. If an animal continued to eat *some* food every day, it eventually showed symptoms resembling polyneuritis. This accords with the findings of Funk (4) and Lumiere (5) that starved pigeons do not develop polyneuritis whereas the birds which continue to eat eventually show the characteristic symptoms of this disease.

The following graphs illustrate the relation of the food intake to the vitamin-B contained in the product used. The maximum ordinate

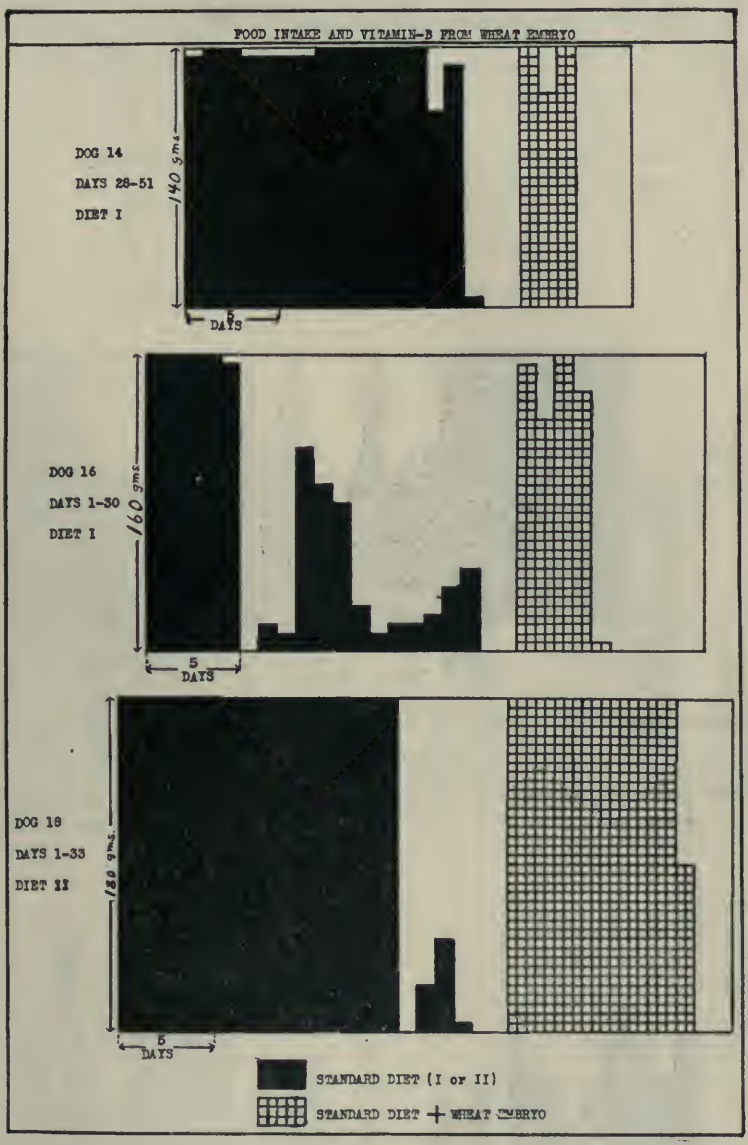
FOOD INTAKE AND VITAMIN-B FROM WHEAT EMBRYO
DOG 14
DAYS 28-51
DIET I
140 gms.
5
DAYS
DOG 16
DAYS 1-30
DIET I
160 gms.
5
DAYS
DOG 18
DAYS 1-33
DIET II
180 gms.
5
DAYS
STANDARD DIET (I or II)
STANDARD DIET + WHEAT EMBRYO

Chart 1

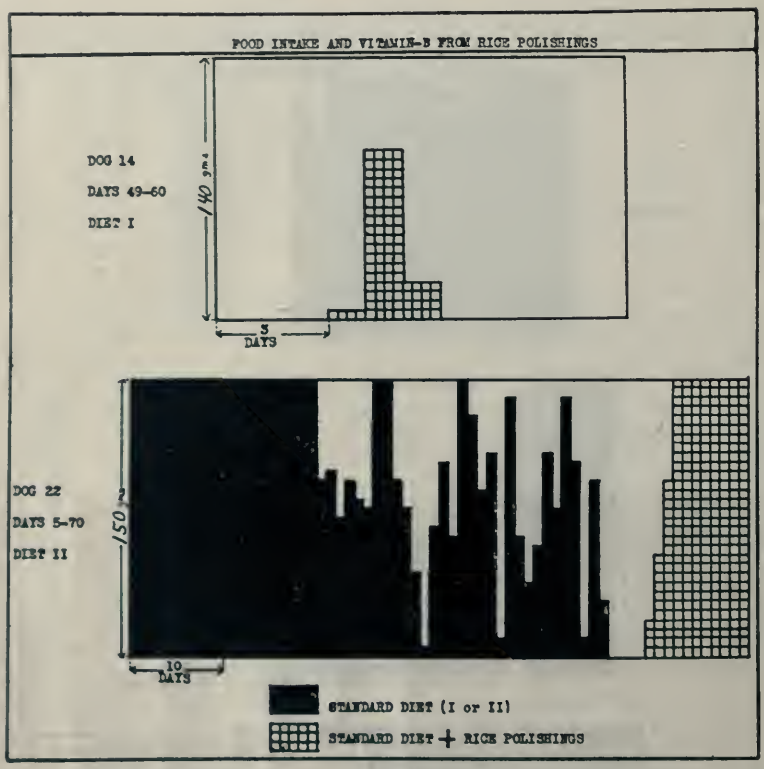

Chart 2

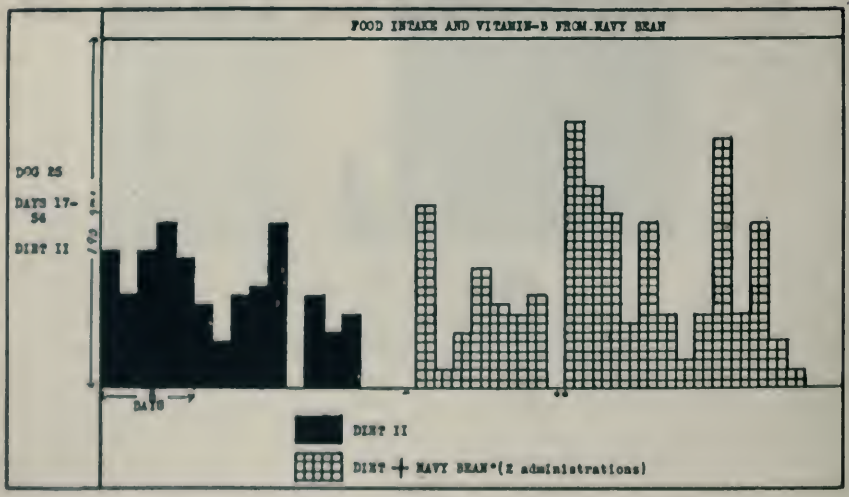

Chart 3

represents the food in grams *offered* to the dog to eat, the quantity being estimated on a basis of from 65 to 80 calories per kilogram of body-weight. In the shaded portion is plotted the *actual* daily food intake.

In testing the various products for their ability to restore appetite it was decided, after some preliminary experience in observing the response of the animals to the food offered, not to administer the vitamin-containing product until the animal had refused the food for 2 days in succession. In many instances dogs refused the food for one day and then began eating of their own accord on the day following the refusal. Chart 4 illustrates this fact.

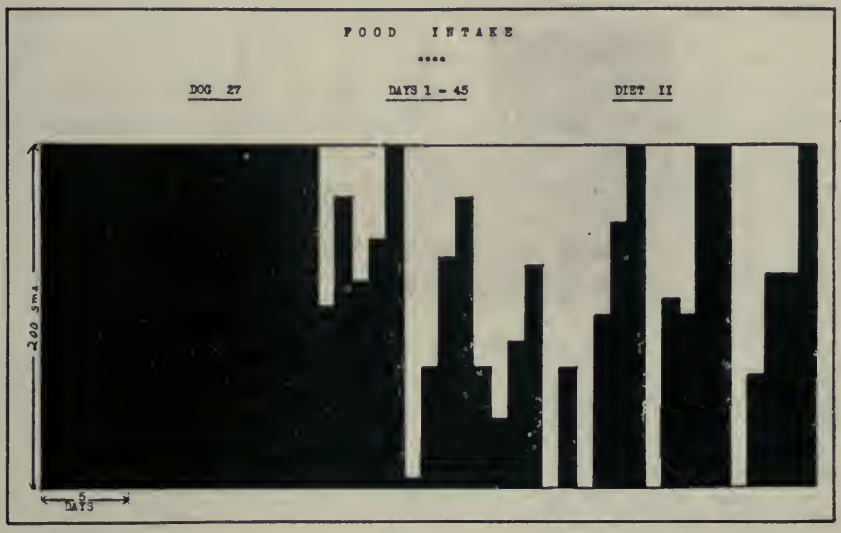

Chart 4

The belief that the product administered supplied a definite stimulus to the appetite and that the recovery of appetite was not purely fortuitous, as the above chart might suggest, is strengthened by the fact that in two cases food which had been refused in the morning was offered again late in the afternoon, several hours after the vitamin-containing product had been given, whereupon the entire amount was eaten within half-an-hour.

Quantitative relationships. It is felt that the number of experiments with these products as sources of vitamin-B is not great enough to warrant any precise statements as to the dog's requirement of vitamin in terms of these sources. The equivalent of 20 grams of ether

extracted wheat embryo was found to be effective for 10 kilogram dogs in every trial that was made. The equivalent of the same amount of ether-extracted rice polishings was also found effective. In the case of ether-extracted navy bean, the administration of the equivalent of 30 grams was followed by a partial recovery of appetite. Tests of these preparations on polyneuritic pigeons showed that the equivalent of 2 grams of ether-extracted wheat embryo or rice polishings sufficed to bring about a complete disappearance of polyneuritic symptoms, while the equivalent of 4 grams of ether-extracted navy bean was required to produce the same result. This parallelism points to the vitamin-B in the products as being the active agent in restoring the appetite.

Pathological symptoms. The symptoms described as developing in response to a deficiency of vitamin-B (1), (2) appeared in ten of the animals used in these experiments. Some of the animals showed a paralysis, which was gradual in its onset and development and which involved only the hind limbs, as the first observed symptom. With most of the animals, however, vomiting appeared as the first symptom. In contrast to the observation of Karr, however, it was not noted that "either the loss of control of the legs or the convulsions may appear first." When vomiting was noticed, the animal was taken out of the cage and allowed to walk about the room. Careful observation usually resulted in the discovery of a slight loss of control of the hind limbs, this being evidenced by a peculiar dragging of the foot and failure to lift it from the floor in a normal fashion. The onset of convulsions occurred in some of the animals more suddenly than in others. The writer believes, on the basis of his own observations, that the convulsions indicate an advanced case of vitamin-B deficiency and that they are symptoms which follow those of muscle spasticity in the limbs with greater or less rapidity.

In many of the animals a foul breath was a very noticeable feature of the syndrome. That an alimentary disturbance occurs in animals suffering from a lack of vitamin-B can hardly be doubted in view of the vomiting and the foul breath. Especially interesting in this connection is the case of dog 25. This animal vomited on the day preceding that on which symptoms of paralysis of the hind limbs appeared. Neutral tomato juice was administered by stomach sound when the nervous symptoms manifested themselves. A large part of the juice was promptly vomited. A small part of a second dose was also vomited. The therapy was continued nevertheless. None of the juice given in subsequent treatments was rejected. All of the symptoms disappeared

in the course of a few days. In the case of dog 23, which was treated with an extract of navy bean, there was a similar inability to retain the material introduced into the stomach. Further evidence that alimentary disturbances accompany or result from a deficiency of vitamin-B was obtained in the experiments with pigeons. The approach of the birds to a polyneuritic condition was in many cases associated with a failure of the crop to empty. The cure of the polyneuritis by injection of vitamin-B was in every case accompanied by the evacuation of a greenish colored fecal mass from the intestine. A similar observation led Voegtlin and Myers (6) to compare secretin and vitamin-B as to both their antineuritic property and their property of promoting the flow of pancreatic juice.

Whether the sympathetic system is affected is a question which naturally arises when the symptoms shown by dog 27 are considered. Figures 1 and 2 show particularly the condition of the hind limbs in this animal. The muscles of both limbs were vigorously contracted and the limbs could be felt as distinctly warm to the touch. Whether this increased heat production in the limbs was merely an expression of a greater volume of blood-flow, or whether it was due to the vigorous and maintained contraction of the muscles was undetermined.

Two of the animals showed paralytic symptoms and a slight incoordination of gait; when they exerted themselves to leave their cages, they were seized with spasms and suddenly died. Heart failure was considered to be the cause of death.

Examination of the leg muscles in those animals which showed paralytic symptoms always revealed a condition of tonic spasticity; the loss of motion in the limb seemed to be due, therefore, not to a degenerative lesion involving the cells of the central nervous sytem, but rather to the presence of some toxic substance. The opisthotonic position which the body assumes during a convulsion and the hypersensitivity of the entire nervous system at such a time recall to mind the symptoms of strychnine poisoning. The tonic spasticity of the limb muscles disappears when the animal is anesthetized by ether, another fact which is in accord with the idea that a toxic substance is responsible for the symptoms which occur in response to a deficiency of vitamin-B.

In this connection it is of interest to note that the researches of Kimura (7) and L'hermitte (8) on the brain and spinal cord lesions occurring in beri-beri show that, so far as the central nervous system is concerned, the histological pictures obtained from cases of beri-beri are indistinguishable from those of toxic polyneuritis. Walshe (9), in reviewing the literature concerning the

Fig. 1. Polyneuritic dog. This animal had been fed for 83 days on a diet lacking vitamin-B. A very slight paralysis involving the hind limbs became evident on the 60th day; on the 74th day the paralysis became pronounced. Photograph was taken on the 83rd day.

Fig. 2. Same animal as is shown in figure 1, photographed on the 83rd day. Notice that the muscles of the hind limb are contracted even when the animal is lying down.

nervous lesion of beri-beri, rejects the Vedder and McCarrison hypothesis that the lesion in this disease is not a peripheral polyneuritis but a degenerative change in the cells of the central nervous system. He also asserts that "so far as is known, the cardiac lesion of beri-beri is identical with that found in diphtheria in which a similar liability to sudden heart failure exists." Walshe favors the toxicity theory of the origin of beri-beri, the idea being that a lack of vitamin-B brings about the production of a nerve and heart poison which is the immediate cause of the symptoms.

The body-weight changes of dogs fed on a diet lacking vitamin-B have evoked some discussion by previous investigators. Voegtlin and Lake (1) emphasize a great loss of body weight as one of the symptoms. Karr (2), on the other hand, claimed that the body-weight changes followed the food intake, and seemed to deny that any appreciable loss in body weight might occur as a result solely of the lack of vitamin-B. The observations made in the present investigation support the contention of Karr. An analysis of the charts, which give the daily food intake plotted along with the body-weight changes, shows that the latter follow the variations in food intake.

The pathological symptoms disappeared after the administration of extracts of wheat embryo or of rice polishings, or of neutralized tomato juice from canned tomatoes. Dog 18, which had been seized with severe convulsions, received the equivalent of 30 grams of ether-extracted wheat embryo by stomach sound within 15 minutes after the first convulsion appeared. Only one convulsion more occurred, about $1\frac{1}{2}$ hours later. Twelve hours after this single treatment the dog was able to walk about the room with only a slight incoördination of gait. Dog 22 became polyneuritic after eating the vitamin-free food for 56 days. Administration of the equivalent of 20 grams of ether-extracted rice polishings produced a distinct therapeutic effect. Treatments with smaller doses were continued for several days and then discontinued. The animal recovered completely from all symptoms of polyneuritis and for 8 days ate the same diet that it had eaten before pathological symptoms had appeared. Dog 26 showed characteristic symptoms of vitamin-B deficiency. First there appeared an incoördination of gait due to a slight paralysis involving the hind limbs. The animal finally became unable to walk and had severe clonic spasms when handled. Figure 3 represents this animal when it was perfectly helpless. The same animal 18 hours later, after it had received neutralized tomato juice, is shown in figure 4.

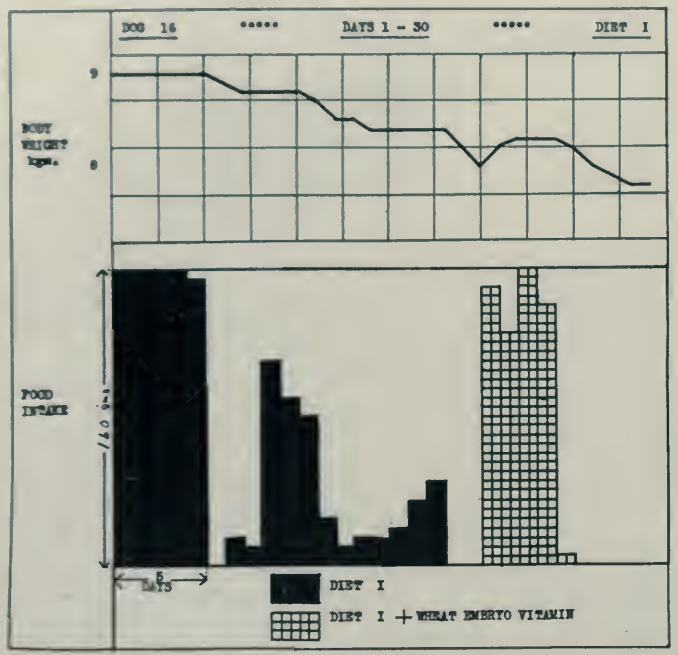

Chart 5

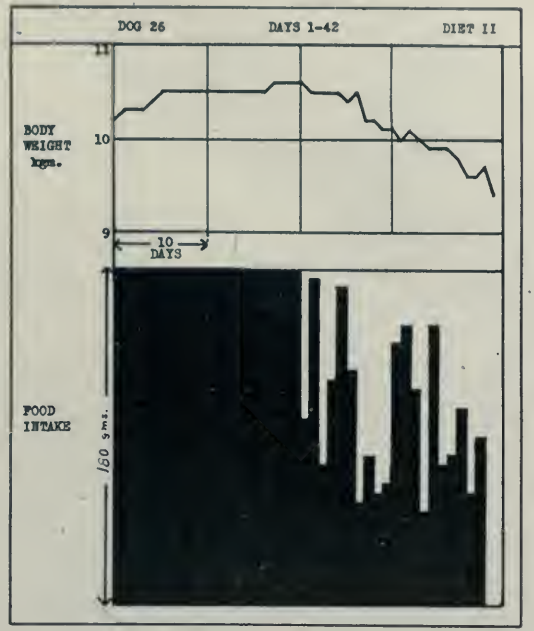

Chart 9

Fig. 3. Polyneuritic dog. The leg muscles were vigorously contracted, especially those of the hinder extremities, resulting in extension of all the limbs and inability of the animal to stand. If this dog was handled, severe clonic spasms resulted.

Fig. 4. Same dog as is shown in figure 3, 18 hours later and showing the effect of administering neutralized tomato juice. After such treatment the animal was able to walk although with a characteristic spastic or "steppage" gait. After repeated treatments extending over 4 days, the spasticity of the leg muscles and spastic gait almost entirely disappeared.

SUMMARY AND CONCLUSIONS

The observation of Karr, that "some relationship exists in the dog between the desire to partake of food and the amount of the so-called water-soluble vitamine ingested" has been confirmed.

To the list of substances demonstrated to contain an appetite-promoting factor, the present investigation has added:

alcoholic extract of wheat embryo,
alcoholic extract of rice polishings and
alcoholic extract of navy bean.

The symptoms of polyneuritis in dogs were made to disappear by the administration of an alcoholic extract of either wheat embryo or rice polishings, or by tomato juice which had been carefully separated from the tomato pulp and neutralized.

All of the products, which were tested and found to restore appetite or to relieve polyneuritic symptoms in dogs, were tested on polyneuritic pigeons, and the birds were cured.

This parallelism indicates that the physiological effects of these products are due to a common factor, probably vitamin-B.

I wish to thank Prof. Lafayette B. Mendel, who suggested this work, for his advice and criticism so freely given.

The expenses of this investigation were defrayed in part by a contribution from the Russell H. Chittenden Research Fund for Physiological Chemistry.

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APPENDIX: A few representative protocols are presented.

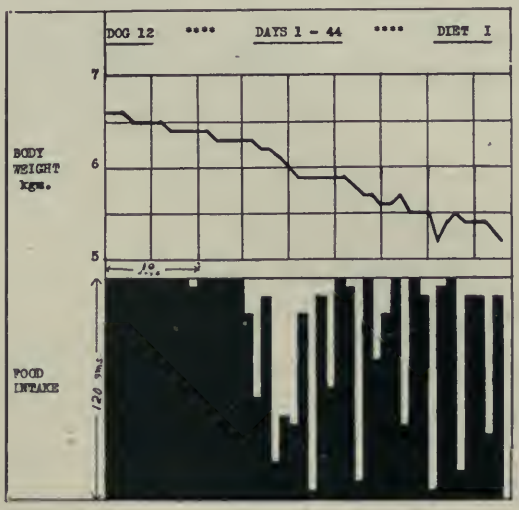

Chart 6

Protocols

Dog 12. The daily food intake and body weight are plotted on chart 6.

33rd day. Some vomitus found in the cage.

43rd day. Animal vomited during the afternoon.

44th day. Animal vomits during the forenoon. Lies down most of the day, which is unusual for this animal. When allowed to walk about the room, animal appears normal. At 7:30 p.m. dog was noticed walking about in the cage with an incoördinated gait; paralysis seemed to be confined to the hind limbs. Placed outside of the cage for closer observation. Animal tried to walk; sprawled out on the floor with clonic spasms lasting about 30 seconds, and died. Heart failure during the spasm was deemed the cause of death.

Dog 18. The daily food intake and body weight are plotted on chart 8.

20th day. Refused the food offered in a.m.

21st day. Refused the food offered in a.m. At 3 p.m. given 20 cc. wheat embryo sample II (= 20 grams) by stomach sound.

22nd to 30th days inclusive. Eats all the food offered. See chart 8.

29th day. Changed from diet I to diet II.

31st day. Eats only 90 grams of food offered.

32nd and 33rd days. Refuses food.

34th day. At noon gave by stomach sound, 3 Harris yeast vitamin tablets in 100 cc. water.

35th day. Refuses food.

36th day. At 10 a.m. gave by stomach sound, 3 grams of dried brewery yeast suspended in 50 cc. water.

37th day. Refused food in morning. At 1:30 p.m. gave 8 grams of dried brewery yeast suspended in 100 cc. water. Offered 180 grams of diet II at 4 p.m. All of it eaten within half an hour.

63rd day. Vomited. Paralysis of hind limbs noticed when animal is allowed to walk about the room. This paralysis is not at all pronounced however.

69th day. Paralysis is more marked than on the 63rd day but is still not very pronounced. Animal does not walk quite normally but drags the hind feet slightly.

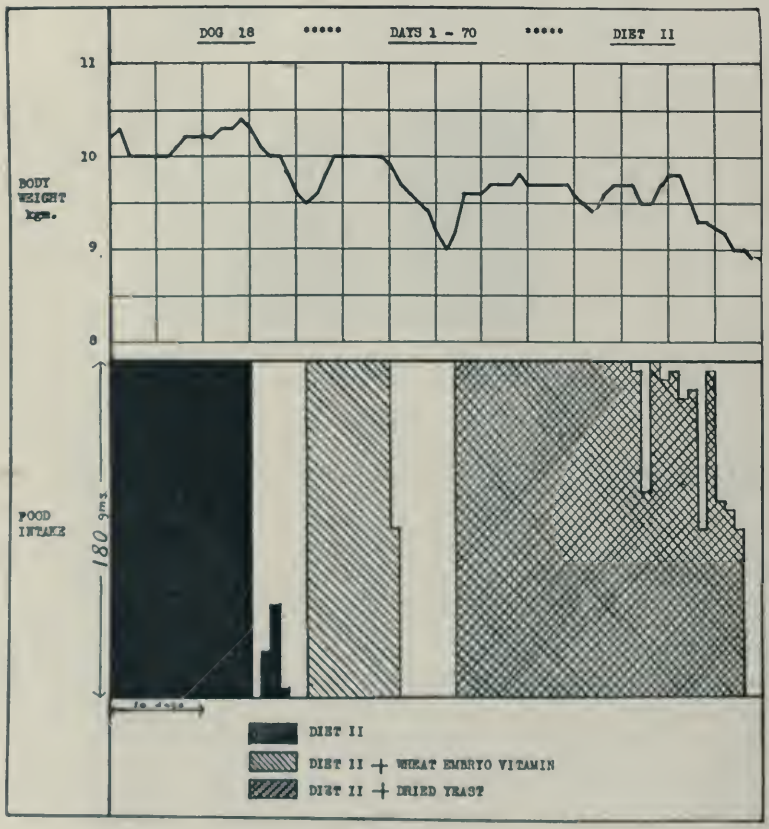

Chart 8

70th day. At 8:50 a.m. dog fell over with convulsions; opisthotonus, and tetanic contractions involving particularly the muscles of the hind limbs. At 9:10 a.m. given 23 cc. of wheat embryo sample III (= 30 grams). Animal has one other convulsion during the forenoon; none during the afternoon. Dog is much improved at 4 p.m. At 10 p.m. the animal was able to walk out of the cage with only a slight wobble in its legs. At 12:30 a.m. of 71st day, animal walked around considerably. At 8 a.m. animal was found dead.

Dog 22. The daily food intake of this animal is plotted on chart 2.

1st day. Offered 120 grams of diet II, which is about 65 calories per kilogram of body weight.

5th day. Animal has not maintained weight satisfactorily on 120 grams of food daily. Therefore increased the amount of food offered to 150 grams.

56th day. Animal shows some paralysis of the hind limbs; favors them when walking about the room.

57th day. Dog is barely able to walk; hind limbs are slightly paralyzed. Muscles of the hind limbs are spastic. At 8:45 a.m. given 10 cc. of rice polishing extract sample II, (= 20 grams).

58th day. Slight improvement in use of hind limbs. At 8:45 a.m. given another treatment with 10 cc. rice polishing extract sample II.

59th day. Limbs are normal but when walking about the room, spasms involving the neck muscles occur and the dog slinks off to the cage to lie down. At 9 a.m. given 20 cc. of rice polishing II (= 40 grams).

60th day. Great improvement over condition on previous day. Given another treatment of 20 cc. rice polishings II (= 40 grams).

61st day. Great improvement. Given 20 cc. rice polishings II (= 40 grams).

62nd to 64th days inclusive. Daily improvement. Given daily treatments of 5 cc. rice polishing II (= 10 grams).

65th day. Good condition. Treatment with rice polishing extract discontinued. (Reference to chart 2 will show that beginning on the 60th day the appetite was restored. On the 63rd to the 70th days inclusive, the animal ate all the food that was offered.)

72nd day. Dog eats only 15 grams. Ate only 40 grams on the 71st day. In hope of restoring appetite, dog was given 10 cc. of rice polishings II (= 20 grams).

73rd day. Dog eats 95 grams of food. At 2 p.m. given 25 cc. of rice polishings II (= 50 grams).

74th day. Dog eats 135 grams of food.

75th day. Eats 200 grams. Increased the amount of food offered from 150 grams in the hope that a restoration of appetite combined with a greater food intake would result in a considerable increase in body weight.

80th day. Experiment concluded. Animal placed on a mixed diet consisting of dog biscuit and meat.

Dog 25. 31st and 32nd days. Dog refuses to eat.

33rd day. Refuses to eat. Is given 15 cc. navy bean extract II (= 30 grams).

34th to 40th days inclusive. Appetite is restored partially (see chart 3).

41st day. Dog refuses to eat. Is given 50 cc. of navy bean extract II (it had been autoclaved for 15 minutes).

42nd to 54th days inclusive. Appetite is partially restored (see chart 3).

55th and 56th days. Dog refused food. Afternoon of 56th day was given 200 cc. of neutralized tomato juice.

57th day. Dog eats 140 grams. Appetite is only partially restored as dog eats part of the food offered up to and including the 63rd day.

65th day. Animal vomited during the night. Walks with a slight loss of control of hind limbs, dragging the feet slightly.

66th day. Dragging of hind feet much more noticeable than on the previous day. Dog vomits a bile-colored fluid during the forenoon. Is apparently very thirsty as it drinks water frequently. At 5 p.m. dog walks with great difficulty

and has clonic spasms when handled. Is apparently in no pain as it wags its tail. At 5.15 p.m. given 200 cc. of neutralized tomato juice. Vomits a large part of it.

67th day. Animal is helpless. Legs are sprawled out (see fig. 3). At 9 a.m. given 200 cc. of neutralized tomato juice. Part of juice is vomited.

68th day. Animal is helpless. Legs are sprawled out as on the previous day. At 8.45 a.m. given 200 cc. of neutralized tomato juice. Photograph taken at 1 p.m. (see fig. 3). Repeated the treatments with neutralized tomato juice at 2:30 p.m. and 8:30 p.m. When observed at 8:30 p.m., the animal was able to walk about the room. It showed a characteristic spastic or "steppage" gait.

69th day. Condition at 9 a.m. is about the same as at 8:30 p.m. the previous day. Photograph taken at 9 a.m. (see fig. 4). Dog eats 25 grams of food. Is given a treatment of 200 cc. neutralized tomato juice at 11 a.m. and another smaller dose of 100 cc. at 5 p.m. Animal shows characteristic spastic gait with some improvement over the previous day.

70th day. Eats 25 grams of food. Spastic gait still very pronounced. At 5 p.m. given 200 cc. neutralized tomato juice.

71st day. Eats 120 grams of food. Spastic gait still evident. At 4 p.m. given 200 cc. of neutralized tomato juice.

72nd day. Eats 170 grams. Spastic gait evident. Given a treatment of 200 cc. of the tomato juice.

73rd day. Eats all the food offered. Spastic gait not so noticeable. Given a treatment of 200 cc. of the tomato juice.

74th to 77th days inclusive. Animal eats all food offered. Experiment concluded on 77th day. Placed on mixed diet. Thirty-seven days later the dog was in excellent condition; there was perhaps a very slight spasticity of the muscles of the hind limbs which was not easy to detect.

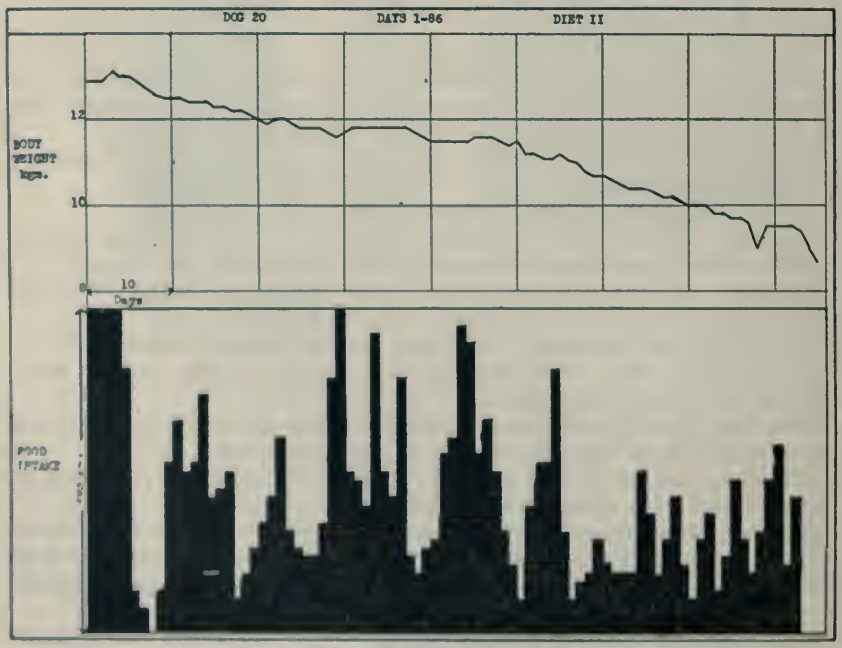

Chart 7

VITAMINE STUDIES

VII. The Influence of Fresh Alfalfa upon the Weight of Testes in Single Comb White Leghorn Cockerels[1]

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Received for publication June 17, 1921

In 1908, Waters (1) published the results of his studies on the influence of under-feeding upon the growth and development of calves. He concluded that skeletal growth will continue at the expense of other tissues when animals are underfed to the point where body-weight fails to increase. Probably the most exhaustive work since 1908 has been that of Jackson and his co-workers, a résumé of which was published in 1918 (2). These studies, as well as those which have been made since 1918 by Jackson (3), Stewart (4), Siperstein (5) and others, show very conclusively that acute inanition is a most important factor in the growth and development of all tissues, especially the endocrine and reproductive organs.

With the advent of chemico-biological methods of research and the recognition of diet as an etiological factor in deficiency diseases, such as scurvy and beri-beri, it was but natural that investigators should turn their attention to problems involving the influence of inadequate diets upon the weight and size of various organs and tissues. It was natural to expect, in the light of more recent work, that investigators should inquire as to the physiological rôle played by each of the recognized food constituents.

Funk and Douglas (6) observed a district atrophy of testes and certain other organs of pigeons when a diet was fed which was deficient in the antineuritic vitamine. Similar observations have been made by Allen (7) and McCarrison (8). The last-mentioned writer has contrib-

[1] Published with the approval of the Director as Paper No. 261, Journal Series, Minnesota Experiment Station.

uted many valuable papers in this field, using pigeons and monkeys as his experimental animals. In his opinion, vitaminic deficiency produces atrophy of reproductive organs with the result that the function of spermatogenesis may be destroyed. Other writers have emphasized the influence of restricted diets upon reproduction in farm animals (9) and albino rats (10).

Hart, Halpin and McCollum (11), Hart, Halpin and Steenbock (12), Lewis (13), Hughes (14) and others have emphasized the importance of vitamines in poultry feeding but, so far as we are aware, little or no work

TABLE 1

Weights of White Leghorn cockerels fed on polished rice

DAY OF EXPERIMENT	WEIGHTS IN GRAMS OF THE COCKERELS									
	No. 264	No. 164	No. 268	No. 453	No. 485	No. 163	No. 452	No. 487	No. 173	No. 189
1	804	811	489	514	793	768	452	800	513	459
4	827	857	503	431	848	822	486	850	529	463
8	852	872	498	457	838	838	469	798	540	475
12	858	898	474	428	840	849	461	800	567	492
16	869	879	472	422	858	850	478	806	566	493
20	880	718	420	392	822	811	481	800	574	517
24	889	638	437	376	848	813	491	805	582	529
28	890	580*	426	300**	821	810	480	800	588	560
30	890	529	426		801	820	481	800	597	574
32	890	†	428		831	797	480	818	600	582
34			411		839	798	495	812	601	587
36			400		856	795	496	822	601	587

* Severe polyneuritis.
** Died, inanition?
† Died.

has been done upon the influence of dietary deficiencies upon the development of the reproductive organs in poultry.

Experimental: The preliminary observations. In our first feeding trials we had occasion to feed two experimental groups of White Leghorn cockerels. The first group received a diet of polished rice, while the second group received a diet of polished rice supplemented with 2 grams of fresh, green alfalfa per day per bird. This work was conducted in the summer of 1919 in connection with our studies of "limberneck" in poultry (15). The experimental birds were single comb White Leghorn cockerels, obtained from a commercial poultry farm, and varied in weight from 450 to 900 grams. No knowledge was available as to their

dietary history with the exception that they had received the ordinary poultry ration employed by the average commercial poultryman.

Ten birds received the diet consisting of rice, while six birds received rice which was supplemented by fresh alfalfa. At the end of the 36-day feeding period the "rice-alfalfa group" (group II) was in much better physical condition, having increased uniformly in body-weight, while the "rice group" (group I) was in much poorer physical condition and some of the birds had developed polyneuritis and died. The growth records of these groups are recorded in tables 1 and 2.

The cockerels in group I, table 1, developed a depraved appetite during the first 20 days of the experiment, eating all of the droppings on the

TABLE 2

Weights of White Leghorn cockerels fed on polished rice and fresh green alfalfa

DAY OF EXPERIMENT	WEIGHTS IN GRAMS OF THE COCKEREL					
	No. 468	No. 182	No. 287	No. 481	No. 462	No. 457
1	540	551	570	808	825	776
4	547	643	604	888	877	789
8	598	624	646	850	863	826
12	610	618	620	898	900	815
16	603	634	615	888	898	791
20	601	634	605	900	902	789
24	628	641	630	902	834	827
28	645	632	652	923	858	847
30	662	642	666	923	860	850
32	670	650	672	913	860	860
34	670	642	680	884	903	858
36	676	652	685	944	912	892

floor of the pen. This was not observed in group II. The latter group was lively and possessed bright red combs and yellow shanks and beaks. The combs, wattles, shanks and beaks of the other group were practically colorless.

On the 32nd day of the experiment cockerels 164, 264 and 488 were caponized and the right testis of each bird was weighed and measured. Cockerel 488 had received a diet of rice and fresh alfalfa. The results are given in table 3.

The apparent effect of such small quantities of green alfalfa is somewhat surprising although Osborne and Mendel (16) have shown that 0.1 gram of dried alfalfa, clover or spinach per day supplied sufficient quantities of the fat soluble vitamine for growing rats. They have

TABLE 3

Preliminary observations on the influence of diet upon the weight and size of testes

DIET	DAY OF EXPERIMENT	COCKEREL NUMBER	WEIGHT OF COCKEREL	WEIGHT OF TESTIS	MEASUREMENT OF TESTIS
			grams	*grams*	*cm.*
Rice only	1	164	811		
	32	164	529	0.0780	1.9 × 0.40
	1	264	804		
	32	264	890	0.1355	1.5 × 0.53
Rice + green alfalfa	1	488	808		
	32	488	919	1.4811	2.2 × 1.60

TABLE 4

Showing the influence of small amounts of fresh, green alfalfa on the development of testes

CHICK NUMBER	GROUP I						
	Rice period				Rice-alfalfa period		
	Body weight 1st day of experiment	Body weight 37th day of experiment	Weight of right testis	Average weight of right testis	Body weight 83rd day of experiment	Weight of left testis	Average weight of left testes
	gram	*grams*	*gram*	*gram*	*grams*	*grams*	*gram*
173	513	601	0.3799		683	1.1398	
487	800	849	0.1008		1004	0.4354	
452	452	498	0.0977	0.1627	578	0.1525	0.5960
189	459	587	0.1848		800	0.6563	
268	489	399	0.0505		Died on 38th day		
	GROUP II						
	Rice-alfalfa period				Rice period		
182	551	652	1.1545		787	0.9455	
468	540	676	1.2905		706	0.2138	
457	776	892	1.3425	1.1944	893	0.1455	0.7382
287	570	685	1.9537		725	1.6481	
462	825	912	0.2308		Died on 52d day		

* When alfalfa is absent testes atrophy. When alfalfa is present the growth of testes is stimulated. The general atrophy of testes in group I cannot be attributed to general inanition for all of the cockerels (on rice diet), with the exception of chick 268, gained in weight.

pointed out, also, that young timothy, clover and alfalfa are richer in the water soluble (B) vitamine than the more mature plants (17).

The limited data of table 3 indicated that the fresh alfalfa possessed the property of preventing atrophy of testes on a rice diet. In order to obtain further information the remaining cockerels were caponized on the 37th day of the experiment, the right testis being removed in each case and weighed.

Immediately after caponizing, the diets of the two groups were reversed, the rice group was given alfalfa, while the rice-alfalfa group was deprived of the alfala. The feeding continued for 46 days, at which time the left testis of each bird was removed and weighed. The data are given in table 4.

The influence of the alfalfa additions (table 4) is quite marked. It is quite apparent that the increased weight of testes in group I on the 83rd day is due to the presence of the alfalfa and not the natural growth, for marked atrophy or cessation of testicular growth occurred in group II as soon as the alfalfa was removed from the diet. Another important fact is brought out in connection with the body-weights of birds throughout the experiment. With the exceptions of cockerels 268 and 462 no losses in body-weight occurred. What seems more remarkable, *atrophy of testes took place while body-weights were actually increasing. This, in our opinion, is evidence that atrophy of organs is not due necessarily to general inanition; neither is it necessary to assume that atrophy of organs is necessarily accompanied by loss of body-weight.* It is quite evident that fresh alfalfa contains something other than protein, fat or carbohydrate which produces a profound influence on the development of reproductive organs. During the 36-day feeding period the right testes of the birds receiving alfalfa (group II) grew practically eight times as fast as those of the cockerels receiving no alfalfa (group I). It is impossible to explain the low weight of testis of cockerel 462, group II. This bird did not grow as rapidly as the others in the group and died on the 52nd day of the experiment period. The atrophy of testes in group II is less pronounced than in group I, due in all probability to a smaller demand for vitamines at the later stages of growth and also to the fact that some vitamine storage might have occurred during the alfalfa feeding period.

Riddle (18) has observed that the right testes of pigeons are heavier, on the average, than the left testes. Our limited data concerning the comparative weights of testes of White Leghorn cockerels do not indicate that striking differences exist although greater differences might be apparent if we had worked upon a larger number of birds. Table 5 gives

the body-weights and testes weights of eight (apparently) normal White Leghorn cockerels.

This would indicate that the weight of testis approximates 1.541 grams per 1000 grams of body weight in birds averaging 1500 grams in body weight. In a later paper we shall describe similar studies in which we have fed purified diets to a larger number of cockerels. In this study protein and the water soluble (B) vitamine have been the varying factors.

TABLE 5

Comparison of weights of testes of normal cockerels

COCKEREL NUMBER	RIGHT TESTIS	LEFT TESTIS	BODY WEIGHT
	grams	*grams*	*grams*
483	1.2300	1.5849	1361
284	1.3560	1.1180	1600
470	0.2705	0.8313	1398
176	0.6370	1.3730	1372
187	2.0931	2.3365	1500
496	3.0215	3.1751	1730
469	6.6547	5.3377	1560
466	3.2875	2.8065	1518
Average.....	2.3187	2.3203	1504.9

CONCLUSIONS AND SUMMARY

The testes of White Leghorn cockerels did not develop when the diet consisted of polished rice. When the rice was supplemented with small amounts of green alfalfa atrophy of testes did not occur and when alfalfa was added to the diets of cockerels which had been on a rice diet for 36 days atrophied testes started to increase in weight.

Atrophy of testes was obtained in cockerels which had actually increased in body-weight, indicating that atrophy of organs is not due, necessarily, to general inanition and loss of body-weight. On account of the small quantities of alfalfa necessary to obtain these results, it would appear that the development of the reproductive organs is dependent, in a large measure, upon the vitamine content of food materials.

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A STUDY OF THE ANTICOAGULATING SUBSTANCES IN THE MUCOUS MEMBRANE OF THE UTERUS

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Received for publication June 21, 1921

The observations outlined in this paper were undertaken in the hope of throwing light on the question of the coagulation of menstrual blood; a problem, according to the literature, on which conflicting opinions are held.

Various theories have been advanced to explain its normal non-coagulability. One, that the alkaline secretion of the uterine cervix is responsible (1) is now regarded as untenable, for the cervical secretion tends rather to favor than to hinder clotting, if added to the blood (2). Neither can it be due to the acid secretion of the vagina—a theory referred to by different authors—for blood collected directly from the cervix exhibits the same property (3). Then too the vagina has no typical glands and in the non-pregnant is kept moist by a small amount of secretion from the uterus (4). A third suggestion has been the presence of an anticoagulating substance in the mucous membrane of the uterus. This theory has been especially developed by Schickele (5), (6). In recent years the problem has been approached along three lines; first, by a comparison of the factors of coagulation in systemic blood taken during the menstrual and intermenstrual phase; second, by an examination of the menstrual blood itself; and third, by observations on the uterine mucous membrane. Birnbaum and Osten (2) studying the question by the first method, added definite quantities of human serum to a fibrinogen solution prepared from oxalated plasma of the horse and found its average time of coagulation, using serum from thirteen cases during the intermenstrual period, to be 44 minutes, while with serum from nineteen menstruating subjects it averaged 75 minutes. They considered the delay due to a diminished amount of fibrin ferment in the blood during menstruation. Rainieri (7), however, could find only slight changes and Kristea and Denk (8), using Wright's method, found no difference in the

coagulation time of blood taken at different periods. These various results were questioned by Cantoni (9), for the data were collected from different individuals for the two periods and in some cases the temperature was not kept constant. In his own studies he used a fibrinogen solution prepared by Schmidt's method and added to it definite quantities of serum from his subjects. He reported no noticeable changes in the coagulation time at different periods. Dienst (10), by a quantitative method, compared the fibrinogen content of menstrual and systemic blood, taken at the same time, and found that the amount was the same in both as it was in the systemic blood taken at other periods. Using Schmidt's method he found less thrombin in the systemic blood taken during menstruation than in that tested at other times and in the menstrual blood the thrombin was diminished to a still greater extent. This led him to the conclusion that there is an anticoagulating substance in the uterine mucosa. Bell (11), since he could not find fibrin ferment in menstrual blood which had passed the vagina, concluded that its precursor had been destroyed in the endometrium. The calcium content of such blood is normal (8) or, according to Bell (12), even above normal. Another series of observations on menstrual blood, collected directly from the cervix, (3) led to the conclusion that it contains thrombin and tends to hasten rather than to retard the clotting of plasma and that its seeming peculiarities are due to the secretions chiefly from the body of the uterus. Recently, Stickel and Zondek (13) state that there are no changes in the coagulation of the systemic blood during the menstrual period and that blood obtained by puncture of the uterus during menstruation clots normally. They conclude that failure to clot is due to an inhibiting substance in the mucous membrane of the uterus. The attempt to find such an anticoagulating substance was made by Kristea and Denk (8) in the course of their work referred to above. But, though they ground the mucosa of a menstruating uterus and extracted it with salt solution, no such anticoagulant was disclosed. The idea was further developed by Schickele (5), (6) when, by means of the Büchner press, he obtained the tissue-juice of a number of organs: human, of the dog, sheep, cow, horse and pig. He tested for the presence of antithrombin by adding definite amounts of the juice to the plasma of the goose and found that from the ovaries and next that from the uterine mucosa produced the greatest delay in clotting, sometimes complete failure to coagulate resulted. This anticoagulant was common to all the animals tested. It was, however, not found in salt solution extracts of the ovary or of

the mucous membrane of the uterus and he is therefore led to conclude that it is closely bound up in the tissue cells. He regarded it as belonging to the hirudin group and thought that it prevented the coagulation of menstrual blood.

From these conflicting reports there seemed need for further consideration of the subject, using the modern methods for the study of coagulation. At Doctor Howell's suggestion, Schickele's experiments were repeated though not with the variety of organs from different animals. Most of the observations were made on the mucosa of the pig's uterus, a few on the uterine muscle and on the ovary of the same animal. The pig was selected because abundance of material could be obtained in all stages of the oestrous cycle and of pregnancy.

Method and observations. The observations were made in three series. Series I during January, February and March, all on the pregnant uterus; series II, from October to March on both the pregnant and non-pregnant and a third during the month of June almost entirely on the non-pregnant organ. In all, twenty-six uteri with embryos varying from a few millimeters to half term, and sixteen non-pregnant were examined. Schickele does not indicate whether his animals were pregnant but he does express regret that he could not obtain material during the period of heat.

The material reached the laboratory from the abattoir while still warm and the mucous membrane was usually dissected off at once from the body of the uterus, though sometimes the uterus remained in the ice-box until the following morning before the dissection was made. The mucous membrane can readily be removed from the pregnant organ and fairly easily from the non-pregnant one, but if the mucosa is not congested the dissection is made with the greatest difficulty. The mucosa was placed in normal saline as it was removed—the process lasting from half an hour to an hour—it was then washed and kneaded by the hands in many changes of sodium chloride, until the last washing was almost clear. The excess of fluid was absorbed by pressing between filter papers and the finely divided tissue either ground with sand in a mortar and then with Kieselguhr or only with the latter. An effort was made to have the specimens of uniform dryness. The material was then folded in a towel or in many layers of filter paper and subjected to a pressure of from three to four hundred atmospheres in the Büchner press. The fluid thus secured was clear with a reddish or sometimes a yellowish tinge. From 1 to 8 cc. were usually obtained, depending on the original amount of material. Preliminary tests were made at once and a more detailed study on the following day.

The fibrinogen solutions, prothrombin and thrombin were all prepared according to Howell's methods (14), and the technique developed by him was employed in the tests. The observations were planned to determine whether antithrombin was present in the pressure-juice, whether its addition to plasma increased the antithrombin time of the plasma, whether an antiprothrombin could be demonstrated by its antagonistic action to prothrombin in solution and by causing delay in the activation by calcium of the prothrombin of oxalated plasma. Each of the forty-two specimens was not, however, subjected to all of these tests.

The evidence for the presence of antithrombin may first be considered. This could be demonstrated in the tissue-juice from the mucous membrane of two of eighteen pregnant uteri tested, while it was present in eight of the fourteen non-pregnant organs. For illustration the following data may be given from one experiment:

A drop of pressure-juice, heated to 54°C. to remove possible fibrinogen, was incubated for 15 minutes with 2, 3, 4 and 5 drops of thrombin; 10 drops of fibrinogen solution were then added.

The times of clotting were:

Thrombin 2, 3, 4 drops, a membranous clot in 6 hours.
Thrombin 5 drops, a membranous clot in 3½ hours.

As a control the pressure-juice was replaced by 0.9 per cent NaCl, and

The times of clotting were:

Thrombin 2 drops, clot in 20 minutes.
Thrombin 3 drops, clot in 10 minutes.
Thrombin 4, 5 drops, clot in 5 minutes.

As was to be expected, when antithrombin could be demonstrated in the tissue-juice, an increase in the antithrombin time occurred, if it was added to oxalated plasma, due to the additional antithrombin in the mixture. But when the juice considered was added to oxalated plasma, which had been heated to 54°C., the coagulation was delayed even more than could be explained by the presence of the added antithrombin. Equal parts of the pressure-juice and plasma were incubated for 15 minutes, then a drop of the mixture was incubated with 5 drops of thrombin for 15 minutes and finally 10 drops of fibrinogen solution added. In 6 hours a slight gelatinous clot had formed, while in the control—in which salt solution was substituted for the juice—a solid clot formed in 20 minutes. Another specimen, in which antithrombin could not be demostrated, when subjected to the same test with oxalated plasma showed the following times of clotting:

Thrombin 2, 3 drops, no clot in 90 minutes
Thrombin 4 drops, clot in 65 minutes.
Thrombin 5 drops, clot in 60 minutes.
The corresponding controls clotted in 30, 30, 20 and 5 minutes.

It is surely of interest that the tissue-juice from sixteen uteri, which did not contain antithrombin, when added to plasma increased its antithrombin time. Three other specimens while containing some antithrombin prolonged still further the antithrombin time of the plasma. The pressure-juice, then, seems to possess the same property as heparin isolated and described by Howell and Holt (15). According to their conclusion, heparin increases the coagulation time by activating the pro-antithrombin in the plasma to antithrombin.

The same workers were able to show that, although heparin has no action on fully formed thrombin, it is able to prevent the activation of prothrombin by calcium. If the uterine "Presssäft" contains heparin or some similar substance, one would expect it to have a similar inhibitory action on prothrombin—preventing or delaying its change to thrombin when calcium is added. To test this point the tissue-juice from twenty-three organs was added to a prothrombin solution prepared from the oxalated plasma of the cat. In ten cases it exercised an undoubted inhibiting action on prothrombin activation—varying from a delay of half an hour in the time of clotting of the fibrinogen solution to failure to clot in 24 hours. A few additional specimens caused slight delay. The data from the tests made on one sample follow—

Prothrombin solution 5 drops added to pressure-juice, 2 drops, incubated 15 minutes, added calcium chloride 2 drops, then fibrinogen 10 drops. Result—Slight membranous clot in 1 hour, not solid in 7 hours.

Control in which 2 drops of water were substituted for the pressure-juice gave a solid clot in 10 minutes.

With another specimen there was a slight membranous clot in 14 hours, while the control was solid in 5 minutes.

The substance causing such delay in the activation of a solution of prothrombin would naturally have a similar action if added to oxalated plasma if the plasma were then recalcified. Thus the specimen referred to above which gave a slight membranous clot in one hour in the prothrombin activation test gave the following results with oxalated cat's plasma:

1 part plasma added to 1 part pressure-juice, then added
$CaCl_2$ 3 drops slight precipitate 40 minutes.
$CaCl_2$ 4 drops slight precipitate 30 minutes.
$CaCl_2$ 6 drops floating clot 30 minutes.
No further change in 12 hours.
When 0.9 per cent NaCl was substituted for the pressure-juice, clots formed in 5, 5 and 2 minutes.

It was much more difficult to demonstrate an inhibiting action on the prothrombin activation of the plasma than on the prothrombin solution. Of those specimens of Presssäft on which both tests were carried out—ten were positive and three weakly positive to the antiprothrombin test. Four of the thirteen were positive to the prothrombin activation test and the others uncertain or negative. However, a positive test was never obtained with the plasma if the prothrombin test was negative.

A few observations were made on the sodium chloride extract of the uterine mucosa and on the ovary. Schickele says that such extracts accelerate rather than retard coagulation. Antithrombin was demonstrated in five of the nine uteri. They all five produced delay in the antithrombin time of the plasma though not more than could be explained by the added antithrombin. It is interesting that the tissue-juice of four of the five mucosa also contained antithrombin. Similar results were obtained on the extract of the ovaries though no observations were made on the ovarian "Presssäft." Antiprothrombin could not be demonstrated in any of the extracts, seeming to indicate that heparin is closely bound up in the tissue cells and cannot be obtained by extraction. On the other hand antithrombin may sometimes be extracted in sufficient quantity so that its effect is not neutralized by the thromboplastic substance present in tissue extracts.

Schickele also obtained an inhibitory substance from the brain, liver, testicle, the suprarenal body, and from the thymus and thyroid—the last two ranking next to the ovary and uterine mucosa in their power to delay coagulation. My observations beyond those on the mucous membrane of the uterus are far too meager to warrant any conclusions but they are, nevertheless, of some interest. The uterine muscle in a few cases was ground and subjected to pressure as described. Great care was taken not to include any of the mucous membrane in the preparation. Although the juice from the mucous membrane of the same organ had in each instance a decided antithrombic action, none could be found in that from the muscle—except in one case in

which the time of clotting was 30 minutes as opposed to 12 minutes in the control. The results on muscle are in agreement with those of Schickele and others. Three samples of the mucosa from the small intestine of the pig were subjected to the same tests as those from the uterus and of these, two showed antithrombin and all three increased the antithrombin time of the plasma. Two increased the prothrombin time of the plasma without delaying the prothrombin activation of a solution of prothrombin. The delay was so marked where obtained that I am inclined to think, with a large series, the results would have been similar to those obtained with the uterine mucosa.

DISCUSSION

An analysis of these observations brings out the fact that a substance which must be fully formed antithrombin may be present in the mucosa of the pig's uterus. In nearly 23 per cent of the specimens, studied its presence was demonstrated in the pressure-juice of the mucosa. It is also sometimes present in saline extract. There is evidence also of the antiprothrombic action of heparin or some similar substance since the antithrombin in heated plasma was increased by a large number of specimens of tissue-juice. Howell has shown that heparin can bring this about by its ability to activate pro-antithrombin to antithrombin and also by being able, further, to prevent the activation of prothrombin to thrombin (15). In this work the second characteristic was repeatedly demonstrated by the use of prothrombin solutions. Evidently Schickele was dealing both with antithrombin and with heparin but by his methods he was not able to distinguish two different substances.

The fact that many specimens of pressure-juice failed to exercise an inhibiting action on coagulation may be due to neutralization of both the antithrombin and antiprothrombin by an excess of thromboplastic substance—always present in tissues and recognized as exerting such action (16), (15). On the other hand, they may be entirely absent from the mucosa due to seasonal variations. However, this does not seem probable for, although antithrombin was found in more specimens examined during June than at other periods, this may have been due to the fact that it was easier to obtain the non-pregnant uterus at this time and consequently attention was concentrated on it. A third possibility is that there are differences due to the period of the oestrous cycle. According to Marshall (17), the domestic sow

is polyoestrous and the duration of the dioestrous phase is from 2 to 3 weeks. During the proestrum there is a sanguineous-mucous flow from the vagina. Corner (18), however, from observations on large numbers of sows, which he knew were in heat, did not confirm Marshall's statement as to the sanguineous flow. He states that the uterine mucosa presents differences in general appearance and texture—"during the oestrous period it is paler than at other times, with a firmer and at times slightly gelatinous inner surface, due to oedema" while a week or ten days later "the mucosa is pink or red, soft and velvety." However, he does not regard the uterine mucosa as a reliable index of the stage of the cycle. No consistent differences could be recognized in the reaction of the pressure-juice of the congested and the non-congested mucosa of the non-pregnant uterus. It is not to be understood, however, that gross examination of them was considered sufficient to place them according to the cycle. Corner suggests that the congestion may sometimes be due to a recent pregnancy or to some pathological condition. Ignorance regarding the age of the animal and of the amount of bleeding of the carcass may be further cause for variation. The only marked difference, then, was between the pregnant and the non-pregnant uterine mucosa and here only in the presence of active antithrombin. In the former the tissue-juice from two of the twenty-six tested had antithrombic action while antithrombin was present in eight of the fourteen non-pregnant ones.

It seems probable from the observations made by Howell and Holt as well as from those recorded on the intestinal mucosa, that the uterine mucous membrane is one of a number of organs from which pro-antithrombin may be obtained as well as antithrombin (15). Schickele reports an anticoagulating substance in the mucosa of the human uterus, taken during menstruation. If heparin and antithrombin are present in the human uterus they might prevent the coagulation of menstrual blood but it is difficult to explain why they should have peculiar significance in the pig's uterus.

Specimens of menstrual blood collected as they passed the vagina are diluted with mucus. An examination of the precipitate obtained when the blood was oxalated and centrifugalized, showed that it was made up of blood corpuscles, bits of fibrin and epithelial cells. Some of the larger masses which had the appearance of clots, when carefully washed, proved to be small pieces of epithelial tissue. Extensive hemolysis had occurred, as was evident in the supernatant fluid. There is no satisfactory explanation of this. The suggestion of Stickel and

Zondek (13) that it is probably the effect of an enzyme does not seem probable. Neither can the hemolysis be due to the action of bacteria, for it has been amply demonstrated, according to Williams (4), that the normal uterus is free from microörganisms. Portions of the fluid were heated to 60°C. in order to detemine whether fibrinogen was present, but with negative results. Clotting did not occur when thrombin was added to such specimens unheated. It has been stated that menstrual blood contains active thrombin, since it will clot plasma (3). In these experiments, however, such results were not obtained either by adding it to fibrinogen solution or to oxalated plasma. This seems strange for if clotting has occurred, as seems evident from the absence of fibrinogen, and the menstrual blood is only serum—then one would expect to find thrombin present. Its presence might be concealed by large amounts of antithrombin but experiment showed that antithrombin was not present in greater amounts than in normal serum. Or it might have combined with antithrombin in the uterus to form the more stable metathrombin. Attempts to determine this point by alakli activation were only partially successful. A precipitate formed when the reactivated serum was added to a fibrinogen solution or to oxalated plasma, indicating a weak thrombic action. In only one instance did a clot form, even after several hours.

The appearance of the menstrual fluid, especially toward the end of the period, suggests old clotted blood in which the clot has almost completely disintegrated. At the beginning the fluid is usually the color of freshly drawn blood, while later it is darker in color and frequently has an odor of decomposition. Possibly the blood clots as it leaves the vessels of the mucosa but locally in small amounts so that no large mass of fibrin is formed and only small fragments of clot, more or less disintegrated, pass from the uterus. If these suggestions are accepted, it is unnecessary to explain the non-coagulability by the presence of an anticoagulating substance in the mucous membrane of the uterus or by peculiarities in the menstrual blood itself.

SUMMARY

1. A pressure-juice obtained from the mucous membrane of the non-pregnant uterus of the pig frequently yields antithrombin. Antithrombin is seldom obtained from the pregnant organ.

2. There is evidence for the presence of heparin—as described by Howell and Holt—in the tissue-juice of the mucosa of both the pregnant and the non-pregnant uterus of the pig.

3. Neither antithrombin nor heparin is obtained with regularity and it is suggested that their presence may be masked by an excess of thromboplastic substance.

4. There is no reason for assuming that these anticoagulating substances have a local function in the pig's uterus, though if present in the human being they might inhibit the coagulation of menstrual blood.

5. Antithrombin and anti-prothrombin were demonstrated in the mucous membrane of the pig's intestine.

6. Neither fibrinogen, thrombin nor antithrombin were demonstrated in menstrual blood. There is reason to believe that it clots as normal blood as it passes the uterine mucosa and that the discharge consists of serum and small bits of clot. There is some evidence that the thrombin of the serum has combined with antithrombin, forming metathrombin.

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THE NATURE OF ALCOHOLIC FERMENTATION

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Received for publication June 21, 1921

In a series of recent papers (1) dealing with studies on the nature of cellular antigens I have presented facts sufficient to warrant the construction of a working hypothesis to the effect that the specific antigens of cells, bacterial and somatic, consist of the various fatty complexes which are peculiar to the different species of cells and which bestow upon their particulate surfaces definite chemical characters.

Every cell is regarded as a colloidal particle presenting a characteristic fatty (in part) surface to its enviroment. The fats of cells, consisting of ordinary fatty acids and their salts and esters intimately emulsified with protein and other material, have been removed from the cells by appropriate chemical methods, their nature and proportions observed, and from these data artificial complexes have been prepared from similar fatty ingredients derived from sources other than the cells in question, and artificial antigens have been made by creating adsorption compounds between the fats and surfaces other than cell stromata. Such aqueous suspension colloids of artificial cells have been used in immunological experiments and have been found to give rise to antibody production and to replace the true cell suspensions in the various serological tests.

As a control experiment to some work on the cells of malignant growths I applied the principles of the hypothesis to the fermentative properties of yeast cells. The results have seemed worthy of the following preliminary note.

To begin with, yeast cells were regarded as particulate entities presenting fairly specific chemical surfaces of emulsified fat. If this fat complex were known and an artificial substitute for it were to be placed upon an appropriate surface and then brought in contact with a solution of dextrose, liberation of CO_2 and the production of alcohol should occur. Accordingly a large quantity of S. cerevesiae was examined and the fats as fatty acids recovered by methods outlined in

previous papers. The identification of the fatty acids constituting the mixture and their proportions showed a not too difficult complex resembling, as Neuss states (2), olive oil. I was unable to verify the statement of Neville (3) that the greater quantity of the acids have the formula C15 H30 O2. Indeed the existence of such an acid with an uneven number of carbon atoms appears doubtful. We were in accord, however, as to the unsaturated fatty acids C18 H34 O2 and C18 H32 O2.

Now, since yeast itself is not highly specific in its fermentation, there being other fungi and perhaps bacteria also capable of inducing alcohol and CO_2, it was thought for the sake of simplicity that a single fatty acid having physical characters approximating those making up the bulk of the complex and fairly well representing it might be employed to advantage. Accordingly oleic acid was determined on, and a sample of perfectly colorless acid having an iodin value of 87.5 and a neutralization value of 140 mgm. NaOH was used.

The question of a proper surface to convey the fat then arose. A variety of substances such as cholesterol, mastic, casein, serum, hemoglobin, agar, etc., in association with 10 per cent dextrose solution, with and without electrolyte and with varying pH, was tried but in no instance was alcohol formed or CO_2 evolved. In this connection it has been stated that many inert substances may bring about fermentation in slight degree. Berzelius (4) mentions animal fibrin, the expressed juices of some plants, cheese, etc., while Guilliermond states that common garden peas immersed in water will cause fermentation by virtue of their surfaces alone. In my hands neither well-washed fibrin (blood) nor fat-free casein alone in contact with dextrose solution, where the mixtures were clear and free from bacteria and fungi, has produced either CO_2 or alcohol even after standing for months at 20°C. Putrid fibrin yields a small volume of gas containing CO_2 but the process is soon checked and alcohol is not formed. The properties of fibrin, however, commended it as a surface suitable for carrying the fats. Therefore 0.250 gram of the substance was ground in a mortar to a granular condition and subsequently allowed to imbibe water in N/20 NaOH under toluene in the ice box overnight. The fluid was then filtered off, the fibrin washed thoroughly in water and the excess of moisture taken up by blotting paper. With the moist fibrin there was then triturated 0.010 to 0.025 gram freshly prepared dry sodium oleate and the mixture placed in the bottom of a chemically clean U fermentation tube, which was then filled with 10 per cent

dextrose solution in distilled water, brought to a pH of 7.6 to 8.3 by NaOH and 0.5 cc. of toluene added. A large number of such tubes, together with various modifications, and of Ehrlenmeyer flasks were prepared and an equal number of controls containing: *1*, 10 per cent dextrose solution alone; *2*, 10 per cent dextrose solution plus 1 agar slant of a 24 hour culture of B. coli; *3*, 10 per cent dextrose plus fibrin; *4*, dextrose plus sodium oleate; *5*, dextrose plus the combined alcohol-ether extracts of yeast; *6*, the same plus fibrin; *7*, dextrose plus fibrin plus the sodium salts of the fatty acid complex derived from yeast; *8*, dextrose plus the above salts alone; *9*, dextrose plus the concentrated aqueous extract of yeast residue after exhaustion in a Soxhlet apparatus with ether and with alcohol for one week each. (This substance still contains yeast fats in considerable amount.)

All mixtures were allowed to stand at room temperature. Control tubes 1 to 6 and 8 remained for months unchanged, sterile and clear save those containing the sodium salts alone with dextrose, which showed slight uniform cloudiness due to diffusion of the salts. After a latent period of a few hours to a day or so the determinant tubes and controls 7 and 9 showed fermentation and the process continued slowly and about equally in all until gas completely filled the long arms. The gas was CO_2 and the fluids gave all the qualitative tests for ethyl alcohol.

The first change to occur in the active mixtures was development of cloudiness of the fluid due to diffusion of some of the sodium oleate; then, quite suddenly, the fluid cleared and at about the same time the fibrin became altered in appearance. Instead of the fibrin grains continuing to be distinctly separate, just touching at points of contact as in control tubes, they became agglutinated into a spongy mat the surfaces and pores of which soon became charged with gas bubbles which not infrequently carried the mat to the top of the long arm of the tube. The use of flasks permitted the aspiration of the gas into quantitative alkali.

These experiments have been repeated many times so that some vagaries of the reaction have been noted. With slight variations in manipulation fermentation sometimes failed, the fibrin did not agglutinate but lost water and looked white and powdery, and its upper surface as well as the wall of the tube was covered with white flocculent oleate. The oleate and the fibrin must combine. In other tubes fermentation would be stormy at first and then slow down perhaps dying out before half finished. Where the proper adsorption was obtained, however, the process was carried on steadily, and in some

cases continued after renewal of the dextrose solution following withdrawal of the gas. Often an inactive tube was activated by shaking, and *vice versa*. In watching fermentation by yeast one is impressed with the fact that the rapidly rising bubbles of gas favor diffusion and hence serve to speed up the rate. The slower formation of gas and its tendency to become held in the mat of fibrin appear to slow the artificial mixtures, so that sometimes a sort of "depolarization" by tapping was necessary to restore the rate. The rate appeared to depend on the proper dose of oleate with reference to the surface of fibrin and upon its optimum adsorption thereon. It was, naturally, always slower than a corresponding weight of yeast, reckoning fibrin and oleate together, but much faster than with a weight of yeast corresponding to that of oleate alone. The best results were obtained with 3 grams of fibrin and 50 mgm. of oleate in a large amount of dextrose solution where 30 cc. of CO_2 were obtained daily for 5 days, or until complete filling of the long arm of a giant U tube occurred.

When acid was substituted for the alkali salt the fermentation often began at once and finished quickly, but in many tubes of all descriptions the rate lagged so that sometimes the process required a month to complete.

The surfaces of such materials as fragments of porous earthenware and of pumice and of common sponge produced no effect on 10 per cent dextrose solutions, but when these were first permeated with films of oleic acid or oleate, the air spaces being well filled, and then brought into sugar solution, fermentation began soon, in some cases within a few minutes, and CO_2 was slowly evolved for variable periods of time, or until the films had entirely separated from the surfaces.

The specificity of oleic acid and its salts is of course not rigid. Impure acids, that is, samples with iodin value of 82, known to contain small amounts of lower saturated acids, and others with iodin value of 95 containing some linolic acid, also produced alcoholic fermentation. Pure linolic acid, one having molecular weight and fluid character similar to oleic but differing widely in iodin value, fermented dextrose equally well. I was unable, however, to obtain fermentation by the saturated acids of the acetic series, such as stearic, or their salts, or from a commercial soap. From the examination of the acids obtained from yeast it was expected that the fluid unsaturated fatty acids would prove most available, and it is possible they may be essential. The salts of such acids are not so readily hydrolyzed in water as are those of the saturated acids, and the acid salts do not separate out save at

temperatures lower than the solidification points of the corresponding acids.

Some samples of lecithin, impure and carrying adsorbed unsaturated acids, also produced alcoholic fermentation in conjunction with fibrin, whereas purified lecithin did not do so.

Without going into detail fully some further observations may be noted. The presence of electrolyte such as phosphate did not appear to be essential although balanced solutions giving a pH about 8.0 were serviceable but no better than those made alkaline with NaOH.

Fermentation appeared to be best between temperatures of 15° and 25°C. Heat, sunlight and extremes of pH outside the limits of 6.5 to 8.5 destroyed the activity. Slator (5) showed that the rate of fermentation by yeast was exactly proportional to the number of yeast cells present. In the experiments here summarized the rate appeared to be proportional, as was suggested, to the extent of film-covered surface, and its maintenance to the ability of the fibrin or other suface to hold the films. With the production of CO_2 and alcohol there was often some acid produced, probably acetic.

Like Buchner's yeast juice, the artificial enzymes fermented other sugars besides dextrose, e.g., saccharose, maltose and lactose, whereas yeast ferments the hexoses only. For some reason no fermentation of levulose was obtained.

Slightly alkaline sugar solutions were found to be fermented most readily, possibly because of the influence of the OH ion in initiating the hydrolysis, but more probably because the initial pH favored the proper adsorptions.

Ten per cent sugar solutions, in distilled water, were used, save in the case of lactose, because it was known that the rate of fermentation is independent of the concentration of sugar between extremes of 1 and 10 per cent (5), and because this concentration favored sterility. Toluene was always used as an added safeguard against contamination. Cultures taken from the fermented mixtures were sterile. The quantities of oleate used were for the most part such as to favor sterility also (6).

In some instances a moderate degree of fermentation was obtained by using, in conjunction with fibrin, the glycerol esters of some unsaturated fatty acids such as are found in olive and in sesame oils.

The production of an artificial zymase similar to Buchner's yeast juice has presented up to the present time insuperable difficulties,

and for this reason it was determined to approach the problem in another way. It was thought that the assumption of yeast juice consisting of colloidal particles of yeast fats, or specific yeast substance, could be sustained if the zymase were to be specifically thrown out of the fluid and rendered inactive by the serums of animals immunized with yeast. To this end rabbits were repeatedly injected with fresh yeast-cell suspensions in salt solutions, and other rabbits with artificial yeast consisting of suspensions of fibrin carrying adsorbed sodium oleate. The serums of normal rabbits were found to contain no agglutinins for yeast suspensions, whereas the serums of the animals injected with the yeast and with the artificial substitute showed agglutinins in dilutions of 1:10 to 1:500, although those artificially induced were not so active as the others. These serums also contained precipitins in comparable quantities as was shown by precipitations with yeast juice (7). Following these procedures, it was then determined that the fermentative power of the precipitated juices had been destroyed. The fermentative power of the cells in the agglutination tests, however, showed an interesting anomaly; those that had been treated with but not agglutinated by normal serums produced no fermentation, whereas the agglutinated cells fermented strongly. At first glance this result was disconcerting but the explanation proved exceedingly simple. Yeast cells, unlike the red blood cell or the cholera bacillus, which easily undergo lysis by immune bodies, are quite resistant in this respect, even in the presence of complement, and preserve their surfaces fairly intact. When treated with normal serum they adsorb therefrom films of a protective colloid which, upon removal of the serum and addition of sugar solution, prevent the latter from reaching the surfaces. The liberation of these films by washing the cells restored the fermenting power. For purposes of illustration the following protocol of a group of animals is given.

Six rabbits of 2 kgm. average weight were selected, of which 2 received six intraperitoneal injections of 3 cc. of a suspension of live yeast cells in salt solution at intervals of 4 days; 2 received similar injections of artificial yeast cells, and 2 were reserved for normal control.

The artificial suspensions consisted of finely gound fibrin, 5 mgm. plus 0.25 mgm. of sodium oleate plus 3 cc. water, pH 8.3. This was allowed to stand with 0.1 per cent trikresol or equivalent for 1 hour before using. Sediment and supernatant fluid were injected together. Like other artificial antigens previously worked with, this showed toxicity, and rabbits were killed by too large doses.[1]

[1] See earlier papers.

Eight days after the last injection all rabbits of the group were bled from the hearts and the serums separated and inactivated at 56°C. for 30 minutes.

To test the agglutinating power of the serums two series of tubes were used, one containing 5 cc. of a heavy opaque suspension of yeast cells in 0.6 per cent salt solution, the other the same quantity of a similar but much lighter and translucent suspension, and to these graduated amounts of the normal and immune serums were added so that dilutions of 1/10, 1/50, 1/200, 1/500 and 1/1000 were obtained. After mixing, these series, together with controls of yeast suspension alone, were placed at 20°C. for 2 hours before readings were taken. Thereafter a small quantity of toluol was added to each tube and both series were let stand in a cool place over night, as so to allow all unagglutinated cells to settle. It was not difficult to distinguish which tubes had flocculated and those in which the yeast cells had merely sedimented, the deposits in the former being of greater volume, white, flocculent and adherent to the sides of the tubes,—the electronegative glass having adsorbed the positive cell-antibody aggregates,—while the latter showed even and level, slimy, buff-colored sediment. All fluids were then carefully pipetted off and replaced by 10 per cent dextrose solution after which fermentation was allowed to proceed at room temperature until finished.

Duplicate sets of tubes were centrifugated following the readings of the result of the agglutination, the supernatant fluid removed, and to each tube 0.5 of a 1/10 dilution of fresh guinea pig serum, or complement, in 3 cc. of salt solution was added. The sediments were then mixed thoroughly with the complement and the tubes placed in the ice-box over night, after which the fluid was pipetted off and 10 per cent dextrose solution added.

The precipitation test was carried out by combining 2 cc. of freshly prepared, clear, filtered maceration juice (and also the aqueous extract no. 9, referred to on page 456) with an equal quantity of each serum to be tested, together with controls of yeast juice alone and serum alone, the mixtures being allowed to stand at 20°C. for 30 minutes. After the precipitates had formed in the tubes containing the immune serums there was added to each tube 1 cc. of a 50 per cent solution of dextrose.

The results of the tests are given in the following table:

TABLE 1

Agglutination

Salt solution suspensions of yeast, 5 cc.

RABBITS	DILUTIONS OF SERUMS			
	1:10	1:50	1:100	1:500
1. Normal rabbit 1	−	−	−	−
2. Normal rabbit 2	−	−	−	−
3. Rabbit yeast 1	++	++	+	+
4. Rabbit yeast 2	++	++	++	+
5. Rabbit art. yeast 1	++	+	+	−
6. Rabbit art. yeast 2	+	+	+	−
7. No serum	−	−	−	−
Fermentation following agglutination				
1	−	−	+	++
2	−	−	+	++
3	++	++	+	+
4	++	++	++	+
5	+	−	+	+
6	+	+	++	++
7	++	++	++	++

Precipitation

Yeast juice 2 cc. Serums 2 cc. Dextrose 1 cc. of 50 per cent solution

1	−
2	−
3	++
4	++
5	+
6	+
7. Juice	−
8. Serum	−

Fermentation

1	++
2	++
3	−
4	−
5	+
6	−
7	++
8	−

++ equals complete agglutination, precipitation or fermentation.
+ equals partial agglutination, precipitation or fermentation.
− equals no agglutination, precipitation or fermentation.

DISCUSSION

The explanation of the foregoing facts is fairly simple. With fibrin and other surfaces on which the fatty acids and their salts were adsorbed I endeavored to make, as well as might be, artificial cell surfaces similar to those of yeast. The reaction of fermentation always occurs at the contact of sugar solution and the yeast mass. This was shown conclusively by Mitscherlich and by Helmholz (8), who separated yeast from sugar solution by animal membranes and found that hydrolysis took place only in that portion of the solution which dialyzed. CO_2 and alcohol were never formed outside the membrane.

Now a mass of yeast culture consists of innumerable individual, minute cell surfaces such that the amount of specific surface is very great in relation to the mass. Immersed in fluid it constitutes a virtual suspension colloid even though the particles quickly settle out. The cell surfaces are specific; that is to say, they are made up of a certain chemical complex which gives them definite special (species) characters. The fats, i.e., fatty acids, salts and esters of yeast are peculiar to the species and differ from the complexes of all other cells I have examined. These fats are largely aggregated at the surfaces of the cells, emulsified with protein, cholesterol or its equivalent, etc., thus constituting a colloid wholly similar to that of the interior of the cells but of reversed type with reference to external phase or content of water, in the sense of Bayliss (9) and of Clowes (10). The presence of unsaturated fat in the membrane causes the cell to retain the Gram stain (11). All the Gram positive bacteria I have examined have shown the unsaturated portion of their fat content to be at least 55 to 60 per cent. The character of the yeast fat in this respect has been mentioned.

The assumption of the presence of the so-called lipoids in the cells appears to be unnecessary, and indeed the existence of such substances in living cells is open to doubt. While it is admitted and believed that very many labile adsorption aggregates of protein, or lower nitrogenous material, fat and electrolyte exist and undergo change with the functional requirements of the cells, it is extremely difficult to understand the reason for the presence or the function of hard-and-fast substances, such for instance as lecithin, the common prototype of lipoids, which are so firm in structure that not even the most powerful reagents are able completely to hydrolyze them. I regard them merely as fortuitous compounds of extraction.

We have then in the yeast-glucose mixture specific particulate or colloid surfaces with an aqueous solution as external phase, and in the artificial mixtures inert membranes, fibrin, clay, etc., bearing adsorption films similar in chemical character to the surface membranes of yeast. Fermentation takes place in both mixtures—in the former briskly, in the latter more slowly, but usually as regularly and continuously. In the artificial fermentation mixtures certain changes take place. Where sodium oleate is used in conjunction with fibrin, a latent period is evident and is required for the proper adsorption to occur. In aqueous solutions the alkali salts of the fatty acids, of oleic less than others, undergo some hydrolysis. If the solution of the salt of a fatty acid is brought in contact with a large surface, the fatty acid set free by hydrolysis "tends to collect in the surface," i.e., it is concentrated or adsorbed there. The hydrolytic equilibrium of the remaining solution is thereby disturbed, and is reëstablished only by the hydrolysis of more salt (12). In this manner continuous films of fatty acid are formed upon the electro-negative fibrin and these surface films act as catalyst. Experience has shown that it is not material whether the sodium salt be rubbed up with the fibrin before the dextrose solution is added, or dissolved in the solution prior to the addition of the fibrin. In the latter case a longer time is required for the adsorption to occur, and sometimes shaking of the mixture is necessary. It is probable that films are formed on the moist fibrin by the trituration so that a shorter interval occurs before fermentation begins, while in the instance where the fatty acid is added to the fibrin in the place of the sodium salt the latent period may vanish and fermentation start at once, although the rate may be very slow, probably because the dose of acid is disproportionately large for the fibrin surface, it being difficult to manipulate small weights of liquid fatty acids. When the glycerol esters of the fatty acids, as in oils, are used with fibrin the small amount of alkali present does not combine with the fat in molecular proportions, but undoubtedly small portions of the alkali salts are formed and these then undergo hydrolysis with subseqeunt adsorption of the liberated acid upon the fibrin. The latent period in such cases appears to be greatest of all.

The mechanism appears to be one simply of catalytic action in which specific surfaces act merely as such without entering into the reaction. At the end of yeast fermentation the cell mass whitens and undergoes change, while in the artificial catalysis the fibrin and fat become dis-

sociated and the mat becomes white and granular. It will be observed that an entirely passive character has been assigned to yeast cells during fermentation. The chief difficulty Pasteur found with the simple equation of Gay-Lussac was that no provision had been made in it toward a supply of oxygen for respiration of the cells. A yeast culture uses some sugar under aerobic conditions and grows rapidly, but yields little fermentation because of the great increase of the mass and the meager amounts of sugar available in shallow depths, whereas under anaerobic conditions in deep containers yeast exerts strong fermentation on pure sugar solutions, remains viable but practically unchanged in mass for a time, and then at length becomes pale, diminishes in volume and disintegrates into a sediment of cellulose free from nitrogen. This was known to Thénard over a century ago. In modern phraseology, the yeast undergoes sterile autolysis. It is evident that for the non-vegetative existence under depths of sugar little oxygen is necessary for the cells to remain viable, and every bacteriologist knows that all ordinary depths of culture fluid contain, to meet such slender demand, abundant oxygen—so much in fact that frequently pieces of sterile animal tissue are purposely placed there to favor anaerobic conditions. There is really no valid evidence to indicate that yeast cells play other than a passive rôle in the phenomena of fermentation.

It is generally admitted that alkaline glucose solutions *tend* to break down spontaneously, and Ducleaux (13) states that sometimes sunlight will start the reaction, yielding in some instances alcohol and CO_2, in others acetic acid. It is probable that glucose is split at once in contact with yeast according to the oldest and simplest equation: $C6\ H12\ O6 = 2C2H6O + 2CO_2$. Since only one intermediate compound is known in heterogeneous systems (14) it appears needless to postulate another in this instance. What doubtless takes place is, according to the conception of Nernst (15), *a*, diffusion of the sugar; *b*, adsorption; *c*, chemical change. Electrolyte, particularly phosphate, is not required. Surface condensation alone is sufficient without recourse to intermediate products or to the idea of some writers, among others Slator (16), that sugar is taken into the cell and passed out as alcohol and carbon dioxide. Such an idea is pure assumption and certainly does not fit the facts in the case of yeast juice, which is cell-free.

If one insists, however, upon the modern chemical hypothesis for the mechanism of fermentation, starting with the well-known effects of

alkali upon dextrose solutions, and following the various steps of reduction by the alternate dissociation and readdition of a molecule of water, it may be assumed that the effect of the catalyst or fat is one which allows such a reduction to proceed in the presence of alkali, whereafter, selecting pyruvic acid as the simplest of several possible substances including methyl glyoxal, glyceric aldehyde and dihydroxyacetone, capable of furnishing CO_2, there remains the oxidation of acetaldehyde to alcohol by the addition of the H, split off from the dextrose molecule at the same time with pyruvic acid, and which is supposed to remain nascent, quiescent and uncombined during the evolution of CO_2. It is argued that this mechanism is rendered more probable by the discovery of the presence of an additional enzyme, carboxylase, in yeast juice. No doubt it will be shown in the future that every change in the colloid state of a sol will give rise to new enzymes, particularly if one is inclined to be satisfied by the explanation of chemical processes through unknown agents.

In the course of attempts to produce a therapeutic substance from yeast by grinding and expressing the juice, E. Buchner (17) experienced difficulty in keeping the product sterile, and to overcome it added concentrated sugar solution, whence came the chance discovery of alcoholic fermentation without the actual presence of yeast cells. From the observations of many workers on the properties of this zymase, or yeast juice, there has been elaborated a most complicated array of esters and aldehydes, enzymes and co-enzymes, chemical reactions and equilibria. Yeast juice is a colloidal fluid invariably contaminated by bacteria, somewhat dark in color, viscous, opalescent, and contains considerable quantities of protein and fat. Its fermentative power is only 1/40 that of an equal weight of yeast, it deteriorates rapidly at 4°C. and is inactivated like the complement of blood serum and like bacterial toxins by filtration through porcelain (the first portion to pass the filter is sterile and retains some fermenting power), by heat, light, shaking, and by adsorption on inert surfaces like charcoal. Without going deeper into the matter at this time yeast juice is a typical emulsion colloid, and yet Buchner and others disregard the fact and attribute its activity solely to its content of "soluble ferment." Unlike yeast, the juice ferments saccharose and maltose as well as dextrose and levulose, requiring the presence of phosphate for the purpose, the real function of which is probably to regulate the pH. While ordinary Berkefeld filtration removes a large amount of the ferment the juice becomes completely inactive on passage through

gelatin filters, one portion of the ferment called the enzyme remaining on the filter, the other called the co-enzyme appearing in the filtrate. Neither is active alone.

Speaking of co-enzyme, Harden (8, p. 4) says:

> The chemical nature and function of this mysterious coadjutor are still unknown, but as it withstands the temperature of boiling water and is dyalizable it is probably more simple in constitution than the enzyme. This, however, is not all; for the decomposition of sugar a phosphate is also indispensable. It appears that in yeast juice, and therefore also most probably in the yeast cell, the phosphorus present takes an active part in fermentation and goes through a remarkable series of changes. The breakdown of sugar into alcohol and carbon dioxide is accompanied by the formation of a complex hexosephosphate, and the phosphate is split off from this compound and thus again rendered available for action by means of a special enzyme, termed hexosephosphatase. In addition to this complex of ferments the cell also possesses special enzymes by which the zymase and coenzyme can be destroyed, and further at least one substance, known as an antienzyme, which directly checks the destructive action. It seems probable, moreover, that the decomposition of the sugar molecule takes place in stages, although much doubt yet exists as to the nature of these.

The hypothesis is too top-heavy to stand. Like Ehrlich's theory of immunity, every weakness demands a new shore until the mass is too unwieldy to work. It is evident the explanation can be made far simpler. Yeast juice is a colloid containing particulate surfaces undoubtedly protein-electrolyte bearing thereon the specific fats of the yeast. In nearly all particulars it bears a striking resemblance to the bacterial toxins, such as of *B. diphtheriae* and others caused by the lysis and disintegration of the germ bodies in a suitable menstruum. These toxins have been shown to consist of colloidal aggregates of the specific fats of the bacterial cells with particles, usually protein, of a certain size, and artificial toxins have been made from similar fat complexes in association with particles of protein, mastic, etc. (18). This is altogether different from the toxin theory of Delbrück and Wortmann (19), who regarded the formation of alcohol by yeast as something similar to the production of bacterial toxins. Colloids similar to yeast juice are obtained by the autolysis of yeast in water as in the active preparations described by Lebedeff (20). With the idea of ferment consisting of these somewhat labile colloidal aggregates it is not difficult to understand the separation of the active principle, co-enzyme, from the particle on which it is carried by filtration through gelatin on which the latter is left behind. In our studies on toxins the dependence of the active principle (fatty complex) on particles of definite size in order to

form toxin was well shown by the expedient of moving the necessary particles from the menstruum (broth) by simple Berkefeld filtration. The addition of the fat complex to unfiltered broth produced toxin, whereas its combination with filtered broth was non-toxic. The bacterial and artificial toxins as well as yeast juice and autolysates are all inactivated by the same agents in the same manner. Harden admits (p. 58) that co-enzyme is a fat, but his comparison of enzyme and co-enzyme with the amboceptor and complement of immune reactions appears to me to be unfortunate since these latter substances do not combine until after the antigen-amboceptor aggregate has formed. Yeast ferment is, however, properly comparable to the antigens, and is indeed antigen, as has been shown in the foregoing experiments. Bechhold (21) states that antienzymes may be produced by injecting animals with the proper enzymes, but gives no reference.

Bayliss (22) on the other hand doubts the specificity of antienzyme, particularly in the case of antiemulsin. While he admits the presence of antibody in the serums of his immunized animals as a precipitin reacting with the "vegetable protein as impurity in the emulsin" used as antigen, he states there was no precipitin for the enzyme itself, and compares the antiemulsin property of serum to the adsorption of trypsin, or rennin, by charcoal. From my point of view yeast is antigen, giving rise to the elaboration of antibody, and the change brought about in the antigen cells, or in the colloidal fluids, by adsorption of specific antibody is fatal to subsequent enzyme action, and it seems immaterial whether one applies the term antienzyme for antibody or not, although if we were to infer that all enzymes were antigens the term would take on specific significance. In this connection I believe that a good share of the discrepancies in the results of the work done on the production of antienzymes is due to the facts so frequently observed in experimenting with cellular antigens and their artificial substitutes, that the same antigen in different colloid states produces in one state excellent antibody and in another little or none, constitutes potent toxin in one and a harmless colloid in another, is strongly hemolytic in the one and nonhemolytic in the other—the one constituting enzyme, the other not at all or suffering change in character, like yeast juice, as its state in the colloid zone is shifted. It is necessary also, in this connection, to keep in mind the fact impressed upon us in our work of the unit character of antibody, which may be in one instance antitoxin, in another a complement fixing substance, or a precipitin, or an agglutinin, according as it operates at one extreme or the other of the colloid realm.

Early in the progress of this work it was thought advisable to conduct somewhat parallel experiments on the enzyme *urease*, but for many reasons the work has not been carried far enough to warrant a report at this time. A number of tentative examinations of the active principle of Jack bean meal, and various trials of artificial fatty complexes comparable to it showed convincingly, however, that definite results had been obtained, and that in all probability urease could be demonstrated to be analogous to the ferment of yeast with respect to its catalytic and antigenic properties.

At the commencement of this paper reference was made to work in progress upon the cells of malignant growths. It will be admitted without question, I think, how vitally essential it is to regard such cells as physico-chemical entities and how necessary to direct study upon their chemistry and upon their colloidal relations and interactions with the normal fluids and tissues of the body from the standpoint of catalytic and immune reactions in order that more light may be thrown upon the obscure problems of sarcoma and cancer.

CONCLUSIONS

It is believed that the experiments summarized in this paper warrant the tentative conclusions that alcoholic fermentation is due to a catalytic process operating at the surfaces of yeast cells, at the colloidal surfaces of yeast juice (zymase), and at artificial surfaces composed of specific fat complexes similar to those found to be present in yeast cells, and that the enzyme of yeast may be regarded as belonging to the cellular antigens.

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HEAT-BLOCK OF SENSORY FIBERS IN THE SCIATIC NERVE[1]

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Received for publication June 23, 1921

The doctrine of specific nerve energy as developed since its early enunciation by Johannes Müller, states that the results of stimulation depend upon the end organ and not upon any peculiarity of the participating neurones. The function of the latter, according to this view, is purely to conduct and so long as they retain this function they are doing all that the animal economy requires.

This doctrine, however, does not imply that all fibers are alike. As an electric conductor might be of platinum or carbon or copper and still conduct electricity so nerve fibers might be conceived as being different in regard to internal structure and nevertheless capable of conducting the nervous impulse. That such differences between neurones actually exist is shown most clearly by experiments upon nervous conductivity. For the conductivity of different sorts of nerves, for example, sensory and motor, may be influenced differently by the application to them of the same agency and differences of this sort can be attributed only to difference of internal structure whether anatomic, physical or chemical.

No attempt will be made at the present time to catalogue the many differences which have been shown to exist between different sorts of nerves either in respect to conduction or in other respects. It is how-

[1] The history of this research is as follows: In 1913–14, Dawson repeated and confirmed the work of Hafemann on the frog; in 1915 Hodges and Dawson extended the work to the dog, cat and rabbit but for reasons mentioned in the text the results were often open to criticism; in 1920 Ostlund and Dawson satisfactorily completed the work on rabbits and the results were embodied in Ostlund's A.B. thesis presented at this University in June of that year; finally a brief summary of the work of Ostlund and Dawon was presented before the American Physiological Society and was published in the Proceedings, This Journal, Vol. lv, no. 2, 1921. Dawson is responsible for the writing of the present article from the proctocols of the experiments.

ever impossible to refrain from pointing out that it is greatly to be wished that some one possessing an adequate knowledge of this subject should bring together for critical examination all the data so far accumulated. A relatively cursory glance on the part of the writer seems to indicate that such a procedure might be very fruitful. He has noted Grützner's observation (1) that of all motor nerves only the vasodilators of the hind-leg are capable of stimulation by heat, and he has felt a thrill of interest on recollecting that these vasodilators, the antidromic fibers of Bayliss, may, on the basis of our present knowledge, be expected to behave in just the perverse way which Grützner described, that is to say, to behave like sensory fibers. But such considerations, captivating as they may be, are beyond the scope of the present report, which has for its purpose to introduce the reader to what seems to be a new specific difference exhibited by motor and sensory nerves in their conductivity when under the influence of heat.

Although our contribution to this interesting subject may be a modest one, we have no hesitation in presenting it since all additions to our knowledge in this field are stepping stones to the comprehension of the nature of the nervous processes and their variations.

Historical. In 1908 Max Hafemann of Leipzig reported his observations (2) on heat block in frogs. He exposed the sciatic, applied to it two pairs of electrodes and then heated the region between these pairs. To avoid the criticism that his stimuli were spreading across the block, he also employed mechanical stimulation in strychninized frogs. As indicators he used the observed movements of the feet, movement on the same side being attributed to direct stimulation, that on the opposite side to reflex stimulation.

He found that a temperature of 44° to 48° caused a disappearance of the conducting power of both sensory and motor nerve fibers of the sciatic but that the power of conduction was first lost by the sensory nerves.

Present Research. *Apparatus and procedure*: The apparatus was simple. A bath kept at a constant temperature held a Mariotte bottle containing physiological saline (0.7 per cent in the case of the frog, 0.9 per cent in that of the mammal). A rubber tube with a transverse hole through it was connected with the Mariotte bottle. This hole transmitted the nerve which was slipped into it by means of a diagonal slit connecting the hole with the outer surface of the rubber tube. Thus the nerve came to fill the hole and lie athwart the lumen of the rubber tube through which flowed the heated saline. The temperature of

the saline depended upon the rate at which it was allowed to flow from the Mariotte bottle, could be regulated by means of a screw clamp and was measured by reading a thermometer inserted into the tube just above the position occupied by the nerve.

On either side of the rubber tube was a pair of platinum electrodes upon which the nerve rested. These electrodes were connected with the secondary coil of an inductorium by way of a commutator which permitted the operator to send the stimulus at will through either pair of electrodes.

The efficacy of any stimulus in respect to the motor fibers was determined by observing the limb supplied by the nerve. The efficacy in respect to the sensory fibers was determined in the case of the frog by the crossed reflex to the other leg, in the case of the mammal by the changes in blood pressure (mercurial manometer) or in respiration. The latter were recorded in the case of the dog by means of an abdominal tambour; in the case of the rabbit by means of a side tube from the tracheal cannula.

The *anesthesia* in the frog was the result of transection of the brain above the medulla and pithing forward, an operation which was performed under ether; in the case of the dog, the anesthesia was due to morphia and ether; in that of the cat to ether only; and in that of the rabbit to a mixture of urethane and chloral given by mouth and to a subsequent light administration of ether.

The experimental procedure consisted in exposing the sciatic, slipping the rubber tube around it, placing the electrodes under it, and stimulating the nerve above or below the part enclosed by the rubber tube both before heating and at intervals during the heating. Between the underlying muscle and the nerve was a thin layer of unheated saline on which the nerve lay except when being stimulated. At such times the electrodes were elevated by the turning of a screw; and, being hooked under the nerve, they brought the latter clear of the liquid before stimulation occurred. The position and fixation of the animal's leg was such as to preclude undue tension upon the nerve.

Results with frogs. The total number of satisfactory experiments performed on frogs was 23. Of these 10 showed definitely a sensory block preceding the motor block; one showed a motor block preceding the sensory while in 12 the observer was unable to secure evidence either way owing at times perhaps to the too rapid heating of the nerve.

The temperatures required for producing sensory block were found to be exceedingly variable as can be seen in the accompanying table (no. 1).

Results on mammals. Two methods were employed in making an examination of the nerve. By one the thresholds of the various responses were determined; in the other the stimulus was constant and the intensity of the responses observed. The latter method requires no further description; the former may be understood from the following example (cf. table 3). If the central electrodes gave a sensory response at 33 cm. (between the coils of the inductorium) and a motor response at 33 cm. and the peripheral electrode gave the same results (at 33 cm. and 36 cm. respectively) and if then, after heating, the former gave such values as 36 cm., 36 cm., and the latter 9 cm., 36 cm., then we may conclude *a*, that the irritability of the nerve above the heated area has remained practically unchanged (now 36 cm., formerly 33 cm.); *b*, that the irritability of the nerve below the heated area has remained unchanged (now 36 cm., as formerly); *c*, that there has been no decrease

TABLE 1

DATE OF EXPERIMENT	MAXIMUM TEMPERATURE	DURATION OF HEATING	AVERAGE TEMPERATURE × TIME
		minutes	
10- 4-13	58	4	335
10- 6-13	55	7	705
9-30-13	53	20	862
2-28-14	52	2	67
9-25-13	52	2	250
10-25-13	47	20	200
3-15-14	47	1	32

in conductivity for impulses passing from the central electrode over the heated area toward the periphery (now 36 cm. and formerly 33 cm.); *d*, that there has been a considerable decrease in the conductivity for impulses passing from the peripheral electrode over the heated area toward the center (now 9 cm., formerly 33 cm.). To this should be added that, as a matter of fact, any central effect produced with the coils at 10 cm. or less is untrustworthy owing to the possibility of leakage of current across the heated area. The block therefore in this illustrative instance is complete in one direction, i. e., in respect to the sensory impulses. The advantage of observing the motor reaction on stimulating with the peripheral electrodes and sensory reaction on stimulating with the central electrodes lies in the fact that they serve as indications of the irritability at the point stimulated. If the sensory response were absent on stimulating the peripheral electrode and at the same time the iritability were low as shown by the difficulty of obtain-

ing a motor response, one would not be justified in concluding that a block had taken place since the absence of a sensory reaction might as easily be due to a loss of irritability at the point stimulated.

As already stated (p. 470, footnote) the experiments on mammals fall into two series. The first of these comprises the work of Hodges and Dawson and the second that of Ostlund and Dawson. To the first belong 8 experiments on the dog, 2 on the cat and 11 on the rabbit; to the second, 11 on the rabbit.

In the *first series* there were a few definite results such as are shown in the following table (figures represent centimeters between coils).

TABLE 2

CENTRAL ELECTRODE		PERIPHERAL ELECTRODE	
Sensory effect	Motor effect	Sensory effect	Motor effect
25	32	35	41 Before heating
28	41	15	40 During heating
30	43	15	42 During heating

Experiment of April 28, 1915 (small dog).

TABLE 3

CENTRAL ELECTRODE		PERIPHERAL ELECTRODE	
Sensory effect	Motor effect	Sensory effect	Motor effect
33	33	33	36
36	36	9	36
36	36	24	36

Experiment of October 6, 1915.

Here a partial sensory block is clearly indicated; indeed the block may have been complete for with the inductorium arranged as in this experiment the stimulating current resulting with only 15 cm. interval between the coils was very strong indeed and probably spread across the heated area.

An excellent example of a sensory block is shown in the accompanying figures (table 3, already referred to) which indicate thresholds in centimeters of distance between the coils.

Here it will be seen that on cooling a partial recovery took place. In this case a positive sensory reaction when the coils stood at 9 cm. was shown to be due to spread of the current from the stimulating electrode.

The experiments of this series were, however, for the most part unsatisfactory for the following reasons. In the first place the nerves of dogs and cats were usually too large to be heated satisfactorily. Secondly, the conclusions drawn from rabbits were vitiated by a silly, experimental error. For, owing to the fact that the respiratory reactions of the rabbits were depressed by too high a degree of anesthesia, the reflex effects upon the circulation came to be recorded instead. A study of the circulatory phenomena led us to conclude that in rabbits the motor block usually preceded the sensory, a belief which was subsequently invalidated by finding that our so-called sensory reaction was due to direct stimulation of the vasoconstrictors of the hind-leg. By the time these errors had been detected, it was found necessary to abandon the research at least temporarily. We mention our troubles for the warning of others.

In the *second series* of mammalian experiments, only rabbits were used, the doses of chloral and urethane were as before but the ether anesthesia was in the successful experiments very light, the respiratory changes were used as indices of sensory function of the nerve examined.

Of these experiments, 8 were for one reason or another unreliable, while the remaining 3, against which we could find no technical criticism, showed indubitably a sensory block which was complete in two cases and partial in the third (exper. July 31, 1920). The values obtained in the experiment of April 19, 1920 are shown in the accompanying table (no. 4).

Here are given for sensory response and motor response the thresholds in centimeters distances between the coils. After determining these thresholds and heating, the coils were set 17 cm. apart. Stimulation then gave a very marked effect except when the sensory fibers were stimulated by the peripheral electrode. The thresholds were then redetermined except in the case of the sensory response to stimulation through the more peripheral electrode. In respect to this last, no effect was obtained until the coils were so near together that escape of the stimulating current could not be excluded. Finally the stimuli at 17 cm. were repeated with the same results as before.

The experiment of July 31, 1920, already referred to as showing incomplete block is interesting also in that it shows the possibility of the recovery of conductivity on cooling. Here the significant values were as follows: central electrode continued to give peripheral responses at 41 cm., meanwhile the peripheral electrode showed first at 21 cm. a marked central response, then two almost negative results (the tem-

perature being 48°), and finally a series of quite positive results. A comparison of the effect upon the respiration of stimulation by the central electrode coil at 21 cm. and that of the peripheral electrode with the coil in the same position, showed marked differences. The stimulus which had to pass the heated area caused an increase in respiratory rate of from 20 (normal) to 24 per 10 seconds and of amplitude of from 10 mm. (normal) to 14 mm. while in the case of the central electrode the corresponding increases were from 20 in 10 seconds (normal) to 24,

TABLE 4

CENTRAL ELECTRODE		PERIPHERAL ELECTRODE	
Sensory effect	Motor effect	Sensory effect	Motor effect
Before heating			
Thresholds			
31	41	35	41
	31		33
	21	33	
23	21	27	29
23		23	23
After heating			
At 17 cm.			
+	+	−	+
Thresholds			
23	27	17−	36
At 17 cm.			
+	+	−	+

Experiment of April 19, 1920.

followed by a marked inhibition (2 and later 4.5 in 10 seconds) and the corresponding amplitudes of excursion of the respiratory tambour were 16, 19, 45 and 45 mm. respectively.

CONCLUSIONS

1. On heating the sciatic nerve of the frog the sensory fibers lose their power of conduction before the motor fibers. (Hafemann is confirmed.)

2. In the frog the temperature required to produce this sensory block is very variable, the average being about 50° (Hafemann 45°).

3. In the frog very rarely the motor fibers are blocked by heat before the sensory.

4. A similar sensory heat block may be obtained in the dog.

5. The temperature at which sensory heat block may be obtained in the rabbit is very variable but may be roughly placed at 50° to 54°.

6. On one occasion, on cooling, recovery of conductivity after sensory block has been observed in the rabbit.

The difference in behavior between the sensory and the motor fibers may be attributed either to differences in the susceptibility of the fibers themselves or to differences in exposure of the two kinds of fibers. It is conceivable that the sensory fibers may lie on the outside of the sciatic nerve and that motor fibers may lie within, so that the former while exposing themselves may protect the latter. Although such a possibility has not yet been excluded by experimentation, it seems highly improbable both inherently and also in the light of what we know of the distribution of the motor fibers in the human sciatic (3), for the latter has been carefully explored by Kraus and Ingham with the purpose of locating the fibers to the voluntary muscles, and there seem to have been no indications that the motor fibers are more centrally placed than the sensory. One may therefore with some justification assume that the results enumerated above are dependent upon a real difference in the constitution of motor and sensory nerves.

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A COMPARISON OF THE RATE OF DIFFUSION OF CERTAIN SUBSTANCES, PARTICULARLY THE FOOD MATERIALS, ENZYMES AND PRO-ENZYMES[1]

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Received for publication May 5, 1921

It is recognized that there is a great difference in the rate at which different materials diffuse through the wall of the alimentary tract into the blood stream. A salt such as sodium chloride, for example, diffuses very rapidly into the blood from the alimentary tract while magnesium sulphate scarcely diffuses at all. It is also known that the rate at which the fatty acids are absorbed is greatly accelerated by the presence of bile.

The present investigation deals with the rate at which several different materials, principally foods, pass through an artificial membrane made of collodion.

Methods and Results. The collodion membrane was prepared from a 62.8 per cent solution of collodion by the method of Beal (1). In brief the method is as follows: This material was poured into a long test tube which was then inverted and allowed to drain. After about 10 minutes the membrane was carefully removed from the test tube and immersed in distilled water where it was allowed to remain until used. All the membranes were made from the same stock solution and in the same manner in order to insure as nearly as possible a uniform thickness. The solution to be dialyzed was introduced into the collodion tube and this was placed in a vessel of distilled water, unless otherwise stated. The names of the substances, the strength of the solutions, and the time of dialysis will be given in the discussion of the tables containing the data.

In table 1 is shown the rate of diffusion of certain monosaccharides, disaccharides, polysaccharides and saccharin. Five grams of each of these substances were dissolved in 25 cc. of distilled water and these

[1] Submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Physiology.

TABLE 1

Table showing the rate of diffusion of the substances named through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Dextrose	5	1.860	37.2	
2	Dextrose	5	1.875	37.5	37.3
3	Dextrose	5	1.845	36.9	
4	Galactose	5	1.765	35.3	
5	Galactose	5	1.795	35.9	35.7
6	Galactose	5	1.795	35.9	
7	Levulose	5	1.730	34.6	
8	Levulose	5	1.700	34.0	34.2
9	Levulose	5	1.705	34.1	
10	Maltose	5	1.395	27.9	
11	Maltose	5	1.415	28.3	28.3
12	Maltose	5	1.430	28.6	
13	Sucrose	5	1.385	27.7	
14	Sucrose	5	1.375	27.5	27.7
15	Sucrose	5	1.380	27.6	
16	Lactose	5	1.220	24.4	
17	Lactose	5	1.220	24.4	24.4
18	Lactose	5	1.225	24.5	
19	Raffinose	5	1.595	31.9	
20	Raffinose	5	1.585	31.7	31.7
21	Raffinose	5	1.575	31.5	
22	Dextrin	5	0.910	18.2	
23	Dextrin	5	0.910	18.2	18.2
24	Dextrin	5	0.915	18.3	
25	Starch	5	0.210	4.2	
26	Starch	5	0.230	4.6	4.4
27	Starch	5	20.15	4.3	
28	Saccharin	5	2.345	46.9	
29	Saccharin	5	2.330	46.6	46.7
30	Saccharin	5	2.335	46.7	

were introduced into the dialyzing tubes which were suspended in vessels containing 1 liter of distilled water each. The materials were permitted to dialyze for 30 minutes. At the end of this time the contents of the diffusion tubes were introduced into a weighing bottle and evaporated to constant weight at a temperature of about 95°C. The decrease in the amount of material in the diffusion tube at the end of 30 minutes was taken as the amount which had dialyzed and is indicated in the table under "grams dialyzed," the original amount being given under "grams used."

It may be seen under "average percentage dialyzed" that at the end of 30 minutes between 34.2 and 37.3 per cent of the monosaccharides had dialyzed; between 24.4 and 28.3 per cent of the disaccharides; 31.7 per cent of raffinose; 18.2 per cent dextrin; 4.4 per cent of the starch; and 46.7 per cent of the saccharin. By comparing these figures it will be seen that the monosaccharides diffused more rapidly than the disaccharides; the disaccharides more rapidly than dextrin or starch and that saccharin diffused more rapidly than any of the substance used.

In table 2 is shown the rate of diffusion of phosphates, carbonates, chlorides, sulphates, citrates, tartrates and acetates. Five grams of each of the substances were dissolved in 25 cc. of distilled water and this was introduced into a dialyzing tube which was suspended in a vessel containing 1 liter of distilled water. The materials were allowed to dialyze for 30 minutes. At the end of this time the contents of the diffusion tubes were removed by several washings with distilled water and were introduced into weighing bottles and evaporated to constant weight at a temperature of about 95°C.

It may be seen in the table that 67.0 per cent of the sodium phosphate had diffused in 30 minutes, 36.6 per cent of the sodium diacid phosphate, and 76.9 per cent of the disodium acid phosphate had diffused in 30 minutes.

By comparing these figures it may be seen that the introduction of one hydrogen atom into the sodium phosphate molecule renders it more diffusible, while the introduction of two hydrogen atoms renders the molecule much less diffusible.

The object of the experiments to be described now is to compare the rate of diffusion of the saline cathartics. It is usually regarded that the cathartics exert their characteristic action by retarding absorption or withdrawing water from the blood by osmosis into the alimentary tract and increasing peristalsis. The three most active saline cathartics are sodium sulphate, magnesium sulphate and sodium potassium tar-

TABLE 2

Table showing the rate of diffusion of the substances named through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Sodium phosphate.....	5	3.350	67.0	
2	Sodium phosphate.....	5	3.340	66.8	67.0
3	Sodium phosphate.....	5	3.365	67.3	
4	Disodium acid phosphate.....	5	3.840	76.8	
5	Disodium acid phosphate.....	5	3.845	76.9	76.9
6	Disodium acid phosphate.....	5	3.845	76.9	
7	Sodium diacid phosphate.....	5	1.825	36.5	
8	Sodium diacid phosphate.....	5	1.850	37.0	36.6
9	Sodium diacid phosphate.....	5	1.820	36.4	
10	Magnesium sulphate.....	5	2.140	42.8	
11	Magnesium sulphate.....	5	2.120	42.4	42.5
12	Magnesium sulphate.....	5	2.120	42.4	
13	Sodium sulphate.....	5	1.840	36.8	
14	Sodium sulphate.....	5	1.840	36.8	36.7
15	Sodium sulphate.....	5	1.825	36.5	
16	Sodium potassium tartrate....	5	3.540	70.8	
17	Sodium potassium tartrate....	5	3.540	70.8	70.8
18	Sodium potassium tartrate....	5	3.545	70.9	
19	Sodium pyrophosphate.....	5	2.805	56.1	
20	Sodium pyrophosphate.....	5	2.815	56.3	56.0
21	Sodium pyrophosphate.....	5	2.785	55.7	
22	Potassium biphosphate.....	5	2.520	50.4	
23	Potassium biphosphate.....	5	2.515	50.3	50.3
24	Potassium biphosphate.....	5	2.515	50.3	
25	Sodium carbonate.....	5	2.865	57.3	
26	Sodium carbonate.....	5	2.870	57.4	57.5
27	Sodium carbonate.....	5	2.890	57.8	
28	Sodium chloride.....	5	3.370	67.4	
29	Sodium chloride.....	5	3.375	67.5	67.5
30	Sodium chloride.....	5	3.375	67.5	

TABLE 2—*Concluded*

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
31	Potassium citrate	5	2.735	54.7	
32	Potassium citrate	5	2.710	54.2	54.3
33	Potassium citrate	5	2.700	54.0	
34	Sodium citrate	5	2.310	46.2	
35	Sodium citrate	5	2.310	46.2	46.2
36	Sodium citrate	5	2.305	46.1	
37	Ammonium tartrate	5	2.485	48.7	
38	Ammonium tartrate	5	2.465	49.3	49.0
39	Ammonium tartrate	5	2.450	49.0	
40	Potassium acetate	5	2.890	57.8	
41	Potassium acetate	5	2.885	57.7	57.7
42	Potassium acetate	5	2.885	57.7	
43	Calcium acetate	5	1.895	37.9	
44	Calcium acetate	5	1.890	37.8	37.8
45	Calcium acetate	5	1.890	37.8	
46	Sodium acetate	5	1.230	24.6	
47	Sodium acetate	5	1.250	25.0	25.0
48	Sodium acetate	5	1.275	25.5	

trate. Hence one would naturally expect these three substances to be less diffusible than the other saline cathartics which are not so powerful in their action.

It may be seen in the chart that the rate at which magnesium sulphate and sodium sulphate diffused was very much the same, being 42.5 per cent and 36.7 per cent respectively, while the sodium potassium tartrate diffused much more rapidly, 70.8 per cent having diffused in 30 minutes.

It may be seen further in the chart that sodium potassium tartrate, a powerful carthartic, is even more diffusible than sodium chloride which possesses very little, if any cathartic action. As a matter of fact, the sodium potassium tartrate was more diffusible than any of the other salts used except the disodium acid phosphate.

It is known that when glycocoll is deaminized a hydroxy derivative of acetic acid is formed and when alanine is deaminized a similar derivative of propionic acid is formed. The rate of diffusion of solutions con-

taining molecular equivalents of these two acids as well as other fatty acids was determined. Twenty-five cubic centimeters of the solution of each acid named in table 3 were introduced into a dialyzing tube and permitted to dialyze against 1 liter of distilled water for 30 minutes. At the end of this time, 1 cc. portion was titrated against N/10 standard sodium hydroxide using phenolphthalein as the indicator. The amounts of the acids dialyzed in 30 minutes are given under "grams dialyzed" in table 3.

TABLE 3

Table showing the rate of diffusion of the organic acids named through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Acetic acid	5.00	2.57	51.4	
2	Acetic acid	5.00	2.54	50.8	51.1
3	Acetic acid	5.00	2.56	51.2	
4	Lactic acid	7.50	3.525	47.0	
5	Lactic acid	7.50	3.600	48.0	47.8
6	Lactic acid	7.50	3.637	48.5	
7	Propionic acid	6.16	2.790	45.3	
8	Propionic acid	6.16	2.796	45.4	45.3
9	Propionic acid	6.16	2.790	45.3	
10	Butyric acid	7.33	2.748	37.5	
11	Butyric acid	7.33	2.748	37.5	37.3
12	Butyric acid	7.33	2.712	37.0	

It may be seen from the table that acetic acid diffused slightly more rapidly than propionic acid and that butyric acid diffused the least rapidly of all the organic acids used.

It is recognized that the presence of bile accelerates the rate of absorption of fats and fatty acids from the alimentary tract. In table 4 it may be seen that bile alone, as well as cream alone, dialyzed through the collodion membrane very slowly, while cream to which bile was added dialyzed rather rapidly.

In the preceding experiments, 25 cc. of bile which had been taken from the gall bladders of yearling calves recently killed at the slaughter house were introduced into the dialyzing tube and dialyzed against 1 liter of

distilled water for 30 minutes. At the end of this time the contents of the diffusion tube were introduced into a weighing bottle as previously described and evaporated to constant weight at a temperature of about 95°C. Twenty-five cubic centimeters of pure cream, and cream containing 5 cc. and 10 cc. of bile respectively, and bile alone were introduced into dialyzing tubes which were suspended in vessels containing 1 liter of distilled water each. The material was permitted to dialyze for 30 minutes. At the end of this time, the contents of the

TABLE 4

Table showing the rate of diffusion of bile and cream through a collodion membrane

EXPERIMENT	SUBSTANCE	PERCENTAGE OF FAT CONTENT			
		Before dialysis	After dialysis	Percentage dialyzed	Average percentage dialyzed
1	Bile	0	0	3.2	
2	Bile	0	0	3.2	3.3
3	Bile	0	0	3.3	
4	Cream	52.0	48.5	7.0	
5	Cream	52.5	49.0	7.0	6.9
6	Cream	54.0	50.0	6.8	
7	Cream 1 cc. bile	54.0	44.0	18.5	
8	Cream 1 cc. bile	53.0	39.5	25.6	22.7
9	Cream 1 cc. bile	54.5	42.0	23.0	
10	Cream 5 cc. bile	52.0	42.0	19.2	
11	Cream 5 cc. bile	54.0	40.0	25.9	22.5
12	Cream 5 cc. bile	52.5	40.5	22.4	
13	Cream 10 cc. bile	49.0	32.5	33.2	
14	Cream 10 cc. bile	49.0	37.0	24.4	28.8
15	Cream 10 cc. bile	48.5	34.5	28.9	

tube were removed and the fat content of each was determined by the Babcock cream tester (2). Similarly, it may be seen in table 5 that the presence of bile increased the rate of dialysis of the two fatty acids, oleic and stearic.

One gram each of the acids and one gram of the acid plus 5 cc. of bile were introduced into 25 cc. of distilled water and this was permitted to dialyze for 30 minutes against distilled water. At the end of this time the material was introduced into a weighing bottle, dried and weighed.

TABLE 5

Table showing the effect on the rate of diffusion of the addition of bile to oleic and stearic acids

EXPERIMENT	SUBSTANCE	GRAMS DIALYZED	PERCENTAGE DIALYZED	PERCENTAGE INCREASE BY BILE
1	1 gram oleic acid	0.023	2.3	
2	1 gram oleic acid	0.026	2.6	
3	1 gram oleic acid 5 cc. bile	0.057	5.7	
4	1 gram oleic acid 5 cc. bile	0.053	5.3	129.1
5	1 gram stearic acid	0.035	3.5	
6	1 gram stearic acid	0.034	3.4	
7	1 gram stearic acid 5 cc. bile	0.062	6.2	
8	1 gram stearic acid 5 cc. bile	0.069	6.9	91.1

TABLE 6

Table showing the rate or diffusion of acetins and glycerol through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Glycerol	5.0	2.595	51.9	
2	Glycerol	5.0	2.620	52.4	52.0
3	Glycerol	5.0	2.580	51.6	
4	Monoacetin	1.25	0.547	43.8	
5	Monoacetin	1.25	0.512	41.0	42.5
6	Monoacetin	1.25	0.532	42.6	
7	Diacetin	1.641	0.555	33.8	
8	Diacetin	1.641	0.576	35.1	34.5
9	Diacetin	1.641	0.568	34.6	
10	Triacetin	2.033	0.252	12.4	
11	Triacetin	2.033	0.246	12.1	12.1
12	Triacetin	2.033	0.243	11.9	

When glycerol is introduced into the alimentary tract, as such, or when it results from the digestion of fat, it is absorbed. It may be seen in table 6 that glycerol dialyzed through the collodion tube very rapidly, 52 per cent having dialyzed in 30 minutes. It may be seen further that

monoacetin, a glycerol derivative with one hydroxyl group replaced by one acetic radicle, diffused less rapidly than the glycerol, and the triacetin, a glycerol derivative with three hydroxyl groups replaced by acetic acid radicles, diffused still more slowly.

In these experiments 5 grams of glycerol were added to 25 cc. of distilled water which was introduced into the diffusion tube and permitted to dialyze against distilled water for 30 minutes. At the end of this time the contents of the diffusion tube were removed and the percentage of glycerol determined by means of the refractometer (3).

One and twenty-five hundredths grams of monoacetin were added to 25 cc. of distilled water and this was introduced into the dialyzing tube which was suspended in a vessel containing 1 liter of distilled water. The material was permitted to dialyze for 30 minutes. At the end of this time the contents of the diffusion tube were introduced into a weighing bottle and evaporated to constant weight at a temperature of about 95°C. Molecular equivalent solutions were made of diacetin and triacetin and the method of procedure was the same as for monoacetin.

During the process of digestion in the small intestines, it is known that a certain amount of soap is formed and absorbed. The object of the experiments about to be described was to compare the rate of diffusion through the collodion membrane of the sodium soaps of butyric, succinic, oleic and palmitic acids. Two grams of each of these soaps were added to 25 cc. of distilled water and this was introduced into dialyzing tubes and permitted to dialyze against distilled water for 30 minutes. The contents of the tubes were then transferred to weighing bottles, dried and weighed. The weights of the dried materials are given in table 7.

It may be seen that 52.7 per cent of sodium butyrate dialzyed in 30 minutes, 36.1 per cent of sodium succinate, 16.7 per cent sodium oleate, and 9.3 per cent sodium palmitate. By comparing the rate of diffusion of sodium soaps of oleic and palmitic acids, the two acids found in ordinary fats, it may be seen that the sodium oleate diffused about 80 per cent more rapidly than the sodium palmitate.

These experiments suggestthat sodium oleate may be absorbed more rapidly from the alimentary tract than sodium palmitate, if they dialyze at the same rate as shown in table 7.

In figure 1 is shown the rate of diffusion of glycerol. The figures along the abscissae indicate the time in minutes, and the figures along the ordinate, percentage of glycerol dialyzed.

TABLE 7

Table showing the rate of diffusion of soaps through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Sodium butyrate.....	2.0	1.050	52.5	
2	Sodium butyrate.....	2.0	1.060	53.0	52.7
3	Sodium succinate.....	2.0	0.730	36.5	
4	Sodium succinate.....	2.0	0.716	35.8	36.1
5	Sodium oleate.....	2.0	0.334	16.7	
6	Sodium oleate.....	2.0	0.336	16.8	16.7
7	Sodium palmitate.....	2.0	0.174	8.7	
8	Sodium palmitate.....	2.0	0.198	9.9	9.3

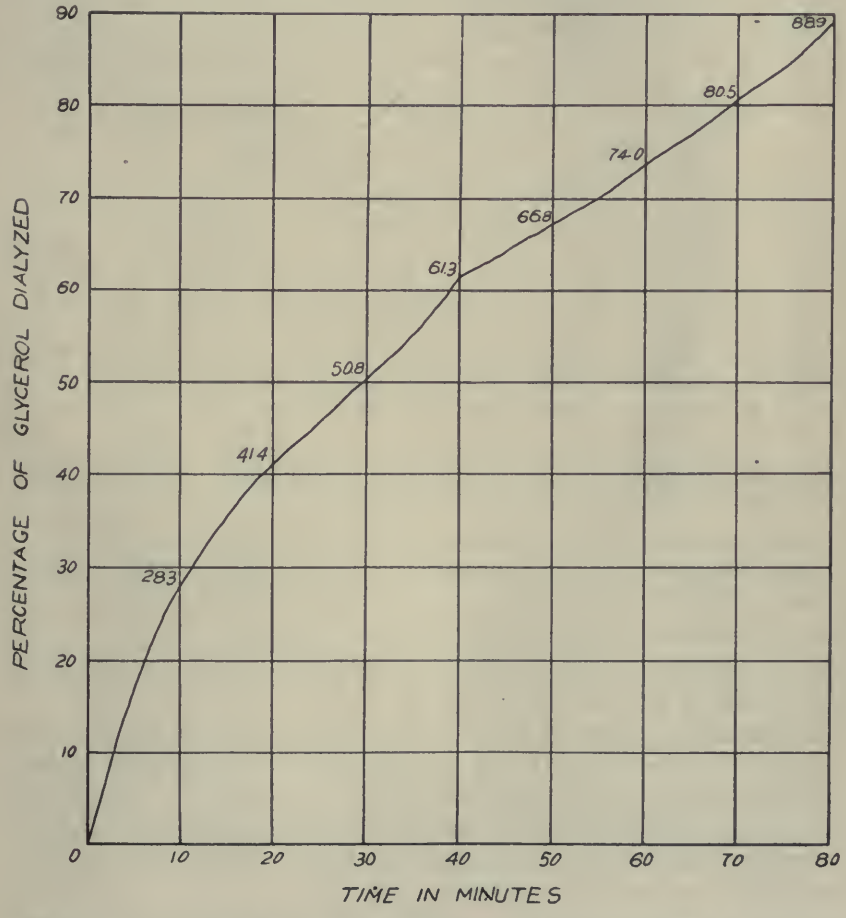

Fig. 1. Curve showing the rate of diffusion of glycerol through a collodion membrane.

It may be seen that 50.8 per cent of the glycerol dialyzed in 30 minutes and that 88.9 per cent had dialyzed in 80 minutes. By comparing the rate of diffusion of the sodium salts of palmitics and oleic acids in table 7 with that of glycerol in figure 1, it will be seen that glycerol diffused much more rapidly than these soaps.

Five grams of glycerol were added to 25 cc. of distilled water and this was introduced into a dialyzing tube which was suspended in a vessel containing 1 liter of distilled water. At intervals of 10 minutes, the

TABLE 8

Table showing rate of diffusion of urea, glycocoll, alanine, aminoids and peptone through a collodion membrane

EXPERIMENT	SUBSTANCE	GRAMS USED	GRAMS DIALYZED	PERCENTAGE DIALYZED	AVERAGE PERCENTAGE DIALYZED
1	Urea	5	4.000	80.0	
2	Urea	5	3.995	79.9	79.9
3	Urea	5	3.990	79.8	
4	Glycocoll	5	2.640	52.8	
5	Glycocoll	5	2.635	52.7	52.6
6	Glycocoll	5	2.615	52.3	
7	Alanine	5	1.975	39.5	
8	Alanine	5	1.970	39.4	39.3
9	Alanine	5	1.950	39.0	
10	Aminoids	5	1.240	24.6	
11	Aminoids	5	1.290	25.8	25.2
12	Aminoids	5	1.265	25.3	
13	Peptone	5	0.880	17.6	
14	Peptone	5	0.890	17.8	17.9
15	Peptone	5	0.910	18.2	

amount of glycerol in the dialyzing tube was determined by means of the refractometer (3).

In table 8 it is shown that the amino acid, glycocoll, diffuses more rapidly than peptone. It may be seen further in the chart that urea was much more diffusible than either of the amino acids. Witte's peptone was used in these experiments and commercially prepared aminoids containing 11.98 per cent nitrogen of which 8.9 per cent was amino nitrogen.

Five grams of each of these substances were dissolved in 25 cc. of distilled water and dialyzed for 30 minutes, evaporated to dryness, and weighed.

In table 9 is shown that the effect of dialysis on the ptyalin in human saliva. Twenty cubic centimeters of saliva were introduced into dialyzing tubes and dialyzed against 1 liter of Ringer's solution for 48 hours in an ice chest. At the end of this time the diastatic power of dialyzed saliva, as well as the non-dialyzed or control, was determined. In all cases of the enzyme experiments to equalize any differences in volume of the solution in the dialyzing tube, the contents of the tubes were diluted to an equal volume before the comparative tests were made. The amount of sugar formed in 5 minutes from the starch paste by 1 cc. of the saliva was taken as a measure of the activity of the enzyme. The starch paste was made by adding 20 grams of corn starch to 500 cc. of

TABLE 9

Table showing the effect of dialysis on ptyalin

EXPERIMENT	ENZYME	DILUTION WITH 0.9 PER CENT NaCl	MILLIGRAMS OF SUGAR FORMED			AVERAGE	PERCENTAGE DECREASE
			Not dialyzed	Average	Dialyzed		
1	Ptyalin	1:0	3.5, 3.5, 3.3	3.4	0.416, 0.421, 0.419	0.419	87.6
2	Ptyalin	1:1	3.24, 3.12, 3.15	3.17	0.335, 0.357, 0.338	0.343	88.8
3	Ptyalin	1:2	2.46, 2.50, 2.38	2.45	0.225, 0.301, 0.243	0.266	48.3
4	Ptyalin	1:3	1.15, 1.16, 1.16	1.16	0.215, 0.220, 0.218	0.218	81.2

distilled water. The mixture was allowed to boil continuously for 20 minutes and then made up to 500 cc. with distilled water. Ten cubic centimeters of the paste were introduced into each of a series of long test tubes, and these were placed in a water bath at 38° C. One cubic centimeter of the saliva was poured into the paste and the tube allowed to remain in the water bath for 5 minutes, when it was run through a free flame and the contents brought to boiling in about 30 seconds, to stop all action of the enzyme. The amount of sugar in 10 cc. of this solution was determined according to the method of Pavy (4). The results are given in table 9.

It may be seen that the strength of the ptyalin was decreased by dialysis, which probably was due to a destruction rather than a dialyzing of the enzyme, since the liquid outside the dialyzing tube possessed no diastatic activity, or the decrease in diastatic activity may have been

TABLE 10

Table showing the rate of diffusion of pepsin, pepsinogen, trypsin and trypsinogen against the solutions indicated through a collodion membrane

EXPERIMENT	ENZYME	DILUTION	SUBSTANCE DIALYZED AGAINST	MILLIMETERS OF EGG WHITE DIGESTED				PERCENTAGE DIALYZED
				Not dialyzed	Average	Dialyzed	Average.	
1	Pepsin	1:0	Ringer's	8, 8, 7, 8	8−	3, 3, 3, 3	3	62.5
2	Pepsin	1:1	Ringer's	8, 8, 8, 7	8−	3, 3, 2, 3	3−	62.5
3	Pepsin	1:2	Ringer's	7, 7, 7, 7	7	2, 2, 2, 3	2+	71.4
4	Pepsin	1:3	Ringer's	6, 7, 6, 6	6+	2, 2, 1, 2	2−	66.6
5	Pepsin	1:0	Ringer's in 0.37% HCl	10, 10, 9, 11	10	9, 9, 29, 8	9−	10.0
6	Pepsin	1:1	Ringer's in 0.37% HCl	10, 9, 11, 8	10−	9, 8, 10, 11	9+	10.0
7	Pepsin	1:2	Ringer's in 0.37% HCl	7, 9, 10, 9	9−	3, 4, 3, 3	3+	66.6
8	Pepsin	1:3	Ringer's in 0.37% HCl	8, 7, 8, 8	8−	2, 2, 1, 3	2	75.0
9	Pepsin	1:0	0.37% HCl	10, 10, 9, 11	10−	10, 9, 9, 9	9+	10.0
10	Pepsin	1:1	0.37% HCl	10, 9, 11, 8	10−	3, 4, 4, 5	4	60.0
11	Pepsin	1:2	0.37% HCl	7, 9, 10, 9	9−	3, 4, 2, 2	3−	66.6
12	Pepsin	1:3	0.37% HCl	8, 7, 8, 8	8−	2, 2, 2, 1	2−	75.0
13	Pepsinogen	1:0	Ringer's	12, 12, 12, 11	12−	12, 11, 12, 12	12−	0
14	Pepsinogen	1:1	Ringer's	12, 11, 12, 12	12−	12, 12, 12, 12,	12	0
15	Pepsinogen	1:2	Ringer's	8, 9, 9, 8	8+	8, 8, 9, 8	8+	0
16	Pepsinogen	1:3	Ringer's	7, 6, 6, 6	6+	6, 6, 8, 6	6+	0
17	Pepsinogen	1:0	Ringer's	9, 9, 9, 9	9	9, 9, 9, 9	9	0
18	Pepsinogen	1:1	Ringer's	7, 6, 7, 7	7−	7, 7, 7, 7	7	0
19	Pepsinogen	1:2	Ringer's	5, 5, 5, 5	5	5, 5, 5, 5	5	0
20	Pepsinogen	1:3	Ringer's	4, 3, 3, 3	3+	3, 3, 4, 3	3+	0

21	Trypsin	1:0	Ringer's	8, 9, 8, 8	8	5, 5, 4, 3	4+	50.0
22	Trypsin	1:1	Ringer's	6, 7, 7, 7	7−	2, 2, 1, 2	2−	71.4
23	Trypsin	1:2	Ringer's	7, 6, 6, 5	6	1, 0, 1, 0	½	90.9
24	Trypsin	1:3	Ringer's	6, 6, 5, 4	5+	0, 0, 0, 1	¼	95.0
25	Trypsinogen	1:0	Ringer's	8, 7, 7, 7	7+	1, 1, 0, 0	½	92.8
26	Trypsinogen	1:1	Ringer's	5, 4, 4, 5	4½	0, 0, 0, 1	¼	94.4
27	Trypsinogen	1:2	Ringer's	3, 3, 3, 3	3	0, 0, 0, 1	¼	91.6
28	Trypsinogen	1:3	Ringer's	2, 2, 2, 2	2	0, 0, 0, 0	0	100.0

due to the inactivation of the enzyme by the membrane. Porter (5) has shown that collodion membranes have the power of inactivating enzymes.

In table 10 is shown a comparison of the rate of diffusion of pepsin, pepsinogen, trypsin and trypsinogen. The pepsin and pepsinogen were obtained from the stomachs of dogs according to the method of Langley and Edkin (6). After removing the stomachs of these animals, slitting them open and washing with tap water, they were immersed in a 1 per cent sodium bicarbonate solution for 1 minute to remove any pepsin adhering. The stomachs were again washed with tap water and rinsed with distilled water. The mucosa was torn off and ground with sand in a mortar. Approximately 200 cc. of 0.7 per cent solution of sodium chloride were added to the finely ground mucosa. The liquid which was pressed out of this material was centrifugalized and filtered through three thicknesses of fine-grained filter paper by means of a vacuum pump. A moderately clear solution was thus obtained which was divided into two portions of 100 cc. each. To one portion was added 1 cc. of a 37 per cent solution of hydrochloric acid while the material was being shaken vigorously. This was the pepsin solution used. Dilutions of 1:0, 1:1, 1:2 and 1:3 were made with 0.9 per cent sodium chloride solution. Twenty cubic centimeters of each of these solutions were introduced into dialyzing tubes and dialyzed in an ice chest against 2 liters of Ringer's solution for 48 hours. Similarly, the diluted pepsin solutions were dialyzed against 0.37 per cent hydrochloric acid and 0.37 per cent hydrochloric acid made with Ringer's solution. At the end of 48 hours, the proteolytic activity of the dialyzed pepsin solutions was determined according to the method of Mett (7). A Mett's tube was introduced into 3 cc. of the liquid and digestion was permitted to proceed for 48 hours at 37°C. The preservative used in these experiments was thymol. At the end of the 48 hours the tubes were removed and the amount of egg white digested was measured. The measurements are given in table 11.

It may be seen that pepsin dialyzed as indicated by the fact that the activity of this enzyme was less after dialysis than before and also by the fact that the solution outside the dialyzing tube contained pepsin.

The 100 cc. of pepsinogen solution made from the gastric mucosa of the dog and freed from the hydrochloric acid and pepsin by means of 1 per cent sodium bicarbonate was the pepsinogen solution used. Dilutions of 1:0, 1:1, 1:2 and 1:3 were made with 0.9 per cent sodium chloride solution. Twenty cubic centimeters of these solutions were dialyzed

against 2 liters of Ringer's solution for 48 hours in the ice chest. The pepsinogen was activated by the use of hydrochloric acid. The proteolytic activity of the solution was determined in the same way as was the pepsin.

It may be seen in the table that the pepsinogen did not dialyze. It may be seen also that trypsin as well as trypsinogen dialyzed but that trypsinogen dialyzed more rapidly than did trypsin.

Pancreatic juice was the trypsinogen solution used. The pancreatic juice to which enterokinase had been added was the trypsin solution.

TABLE 11

Table showing the digestive activity of the solution outside the dialyzing tubes which contained the enzymes, pepsin, trypsin and trypsinogen respectively

EXPERIMENT	ENZYME	SUBSTANCE DIALYZED AGAINST	MILLIMETERS EGG WHITE DIGESTED			
			Normal	Average	Dialyzed Material	Average
1	Pepsin	0.37% HCl	10, 8, 9, 8, 9	9	3, 3, 4, 3, 3	3
2	Pepsin	0.37% HCl	8, 9, 7, 8, 8	8	3, 3, 2, 3, 4	3
3	Trypsin	Ringer's	10, 9, 11, 11, 12	11	2, 1, 2, 3, 2	2
4	Trypsin	Ringer's	9, 10, 11, 8, 9	9	2, 2, 2, 1, 3	2
5	Trypsinogen	Ringer's	10, 9, 9, 8, 8	9−	2, 1, 2, 2, 1	2−

TABLE 12

Table showing the effect of dialysis on catalase

EXPERIMENT	ENZYME	OXYGEN NOT DIALYZED	LIBERATED DIALYZED	PERCENTAGE DIALYZED
		cc.		
1	Catalase	500, 500	495, 500	0
2	Catalase	450, 450	455, 450	0
3	Catalase	380, 365	380, 375	0

The juice was obtained according to the method of Starling (8). The proteolytic activity of this juice was tested by the use of Mett's tubes. Twenty cubic centimeters of trypsin as well as 20 cc. of trypsinogen were dialyzed against 2 liters of Ringer's solution for 48 hours.

In table 12 is shown the effect of dialysis on catalase. The catalase solution used was the defibrinated blood of a rabbit which was diluted 1:6 with 0.9 per cent sodium chloride solution. Ten cubic centimeters of this blood were dialyzed in an ice chest against 3 liters of 0.9 per cent sodium chloride for 48 hours. The catalytic activity was determined

before as well as after dialysis. The determinations were made by adding 0.5 cc. of the blood to neutral hydrogen peroxide at approximately 22°C. in a bottle, and the amount of gas liberated in 10 minutes was taken as a measure of the amount of catalase in the 0.5 cc. of the blood. The results of these determinations are given in table 13. It will be seen that catalase did not dialyze through the collodion membrane.

No attempt will be made in this paper to discuss the large literature relative to the theories of absorption and secretion that has been stimulated by the controversies based on the two theories. Suffice it to say that the facts accumulated do not seem sufficient to demonstrate conclusively one view or the other.

The experiments bearing on the subject reported in this paper would seem to favor the mechanical theory. It may be recalled, however, that in this paper all of the constituents of the urine, organic as well as inorganic, urea was found to be the most diffusible.

Smétanka (9) showed the presence of pepsin in the urine. That this pepsin comes from the stomach is shown by the fact that this enzyme disappears from the urine upon removal of the stomach from the animal. The fact that the pepsin comes from the stomach shows that the enzyme must have diffused through the cells of the walls of the stomach as well as the cells in the kidney. The experiments reported in this paper show that pepsin is diffusible while pepsinogen is not. This observation suggests that the passage of the pepsinogen from cells of the gastric glands into the stomach is a true secretory process and not one of osmosis and diffusion. Buchner and Rapp (10) showed that maltase is diffusible, while it was shown by Brown and Morris (11) that zymase is not. It was also found that both trypsin and trypsinogen diffused through the collodion membrane, the trypsinogen diffusing more rapidly.

It was hoped that the study of the diffusibility of the materials named in this paper might throw some light that could be applied to diffusion through a living membrane, but any conclusions on this point require further work.

SUMMARY

1. The addition of bile to neutral fats as well as fatty acids increased the rate of their diffusion through a collodion membrane, just as it increases the rate of their absorption from the alimentary tract. Glycerol diffuses more rapidly than either oleic or palmitic acids or the sodium soap of these acids.

2. The monosaccharides diffuse through the collodion membrane more rapidly than the disaccharides and these more rapidly than the polysaccharides.

3. As a rule, the more powerful saline cathartics diffuse less rapidly than the less powerful ones. The conspicuous exceptions to this rule suggest that there are factors involved in the action of these cathartics other than simple diffusion and osmosis.

4. The same amino acid, glycocoll, diffuses more rapidly than alanine; and acetic acid, a substance closely related to glycocoll, is more diffusible than propionic acid, a substance closely related to alanine.

5. The enzymes ptyalin and catalase are not diffusible through a collodion membrane. Pepsin is diffusible while pepsinogen is not. Both trypsinogen and trypsin are diffusible, the trypsinogen being the more diffusible.

6. Of all substances used in this investigation, urea was found to be the most diffusible.

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