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THE RESPIRATORY DEAD SPACE

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A correct estimation of the volume of the respiratory dead space is of fundamental importance in connection with many of the problems of the regulation of breathing and related topics. The interpretation of the data accumulated by many investigators depends upon the decision of the question whether the dead space is a fixed or a variable quantity. If the latter is the case, what are the nature, cause, and mode of control of such variations?

Although earlier investigators recognized the dead space as a factor in breathing,[1] Loewy,[2] seems to have been the first to report a measurement of it. By means of a plaster cast of the cavity of the mouth, pharynx, trachea, bronchi and bronchioles of a cadaver he found a volume of 144 cc. He noted, however, that in the living subject even in an expiration much smaller than the volume of the dead space there are always to be found considerable amounts of CO_2, and that as expiration progresses the percentage of CO_2 in successive portions increases gradually, not abruptly: facts which later investigators have not always kept in mind.

Basing their opinion upon these observations, Zuntz and his collaborators in their extensive investigations have assumed the dead space to be an unvarying volume—the same no matter whether the subject were at rest, or breathing deeply from physical exertion, or on a mountain. All of their calculations of the alveolar ventilation and of the composition of the alveolar air involve this assumption. The method employed by them was based on the proportion: the composi-

tion of the alveolar gases (i.e., the excess of CO_2 over, or the deficiency of oxygen under, the inspired air) is to the composition of the (total, mixed) expired air as the tidal volume is to the tidal volume minus the dead space. It is noteworthy that by this method the constancy of composition of the alveolar air was not discovered. As Haldane and his co-workers have demonstrated that such a constancy normally exists, the natural inference is that the dead space is of widely varying volume.

This inference was followed up by Douglas and Haldane[3] who found when the subject was at rest a volume of 160 cc., and a progressive increase with physical exertion up to more than 600 cc. Their method was based (as all indirect determinations must be) upon the same proportion as that used by Zuntz, Loewy and others, but with this difference that Douglas and Haldane, instead of assuming the dead space and calculating the alveolar gases, have determined the composition of the alveolar air by direct analysis, and calculated the dead space. The other terms in the proportion are the (mean) tidal volume as determined from the total volume of air expired into a Douglas bag in a certain number of breaths, and the composition of this expired air.

Even before this work of Douglas and Haldane, Siebeck,[4] had found that during the hyperpnoea of physical exercise the dead space is increased. He used a method involving only a single breath—instead of the average volume of a series of expirations—in which 500 cc. or 1000 cc. of hydrogen were inspired, and then expired into a gasometer. The percentage of hydrogen in the alveolar air (from a sample at the end of expiration), the percentage in the mixed expired air, and the volume of the expiration afforded three terms in the proportion from which the fourth, the dead space, was calculated. He concluded that the dead space is larger with the lungs inflated, and suggested that this might be due to participation of the bronchi in the respiratory movements. Thus far, as we believe, Siebeck was correct. He concluded also, however, that the depth of breathing does not influence the dead space, and that it is even decreased during the deep breathing caused by inhaling CO_2. On these points we think that Siebeck was misled by not using sufficiently deep inspirations.

The latest considerable contribution to the problem of the dead space has been made by Krogh and Lindhard.[5] They have returned emphatically to the doctrine of a dead space of unvarying volume. They first subjected the method of Siebeck to a decidedly damaging criticism, and then adopted it themselves and founded quite precise conclusions

upon it. They have recently published a number of papers containing a large amount of data on various respiratory problems. In the interpretation of their data they have used a fixed dead space, and, as they themselves have remarked, if the dead space is a variable they will have to recalculate all of these experiments, and reconsider their arguments.

As we shall show that the dead space is a variable, it is of interest to note how Krogh and Lindhard were caught in a trap set (quite unintentionally) by Douglas and Haldane. The latter suggested that the enlarged dead space, which they found during hyperpnoea, serves to facilitate the flow of air, and is due to an active broncho-dilatation under the influence of nervous or chemical stimuli.[6] This highly teleological suggestion has not, so far as we can discover, led Douglas and Haldane into any error of fact, but it has led Krogh and Lindhard to confuse the question of an augmented dead space with the quite distinct problem of an active broncho-dilatation.

Accordingly, Krogh and Lindhard, in testing the volume of the dead space at rest and after exercise, seem to have arranged intentionally that the subject should take no deeper inspiration under one condition than under the other. The two measurements were equal; and they concluded therefore (correctly) that there is no evidence of active, teleological, broncho-dilatation during exercise. They did not, however, make adequate tests of the volume of the dead space in breaths as deep as occur during hyperpnoea. If they had, they would doubtless have found, as we do, a dead space varying up to as great a maximum as Douglas and Haldane claim.[7]

The true explanation of these variations is in the main, we believe, that during extra deep breathing the bronchi and bronchioles are dilated passively with the rest of the lungs. Thus a deep inspiration involves an increased dead space no matter whether the subject is at rest or exercising. We recognize, of course, that broncho-dilatation and constriction are functions under sympathetic or autonomic control, that they are influenced by drugs, and are subject to pathological disturbances.[8] Our results demonstrate merely that the ordinary variations of the dead space as between quiet and deep breathing are not for the most part of this active character.

Axial flow of gases through the tubes. As several of the recent investigators in this field have evidently left out of account the peculiarities of the movements of liquids and gases in tubes, we may begin the report of our data with a few simple experiments on this topic. They illus-

trate why it is that with a dead space of 150 cc. the first 150 cc. expired (or even the first 50 cc.) are not free from a considerable admixture of pulmonary air. They afford the reason why a dog during heat polypnoea may have a tidal volume considerably less than the volume of the dead space. They suggest that, during rapid shallow breathing in man, what we may call the physiological dead space is a much smaller volume than the anatomical dead space of Loewy's plaster cast. There may easily be a gaseous exchange sufficient to support life even when the tidal volume is considerably less than the dead space.

If one takes a glass tube of, say, a meter length and one or two centimeters bore, and blows tobacco smoke into one end, he sees that the smoke does not move along the tube in a cylindrical column, filling the tube from side to side, but in the form of a very thin spike. If the tube is held vertically, so that gravitational effects are avoided, the spike follows the axis of the tube, and the tip of the spike begins to issue from the upper end before the tube as a whole is more than a quarter or a third filled with smoke. The quicker the puff, the thinner and sharper the spike, and the more smoke must be blown through before all the clear air is washed out.

If, at the moment that the tip of the spike reaches the upper end of the tube, the puff is stopped by applying the tongue to the lower end, the spike breaks instantly everywhere; and the tube is seen to be filled from side to side with a mixture of smoke and air, thin at the upper end, a thicker mixture at the lower, and all gradations between.

If now an inspiration is made, a thin spike of clear air projects itself down along the axis and into the mouth of the operator along with some of the thicker mixture in the lower end of the tube.

An even more striking demonstration is obtained with a glass bulb having inlet and outlet tubes on opposite sides. When smoke is blown in through one of these tubes, the column at first shoots across the centre of the bulb and out through the other tube with little contamination of the clear air surrounding the stream. If it is stopped suddenly, a complete mixing of smoke and clear air occurs almost instantaneously, and thereafter a very large volume of air must be drawn through the bulb before the last trace of smoke is washed out.

For instrumental purposes these frictional effects are easily neutralized by filling the large glass tube with some loose fibrous material such as glass wool, jute, disks of wire gauze, etc. The friction being then the same at every point in the cross section, the column of smoke pushes the air ahead of it like the plunger of a pump. This holds true even

when the fibrous material is so loosely packed as to afford no noticeable resistance to an expiration.

We have employed a tube of this sort to determine the composition of successive fractions of an expiration. At intervals along the tube were inserted side tubes to which were connected small pipettes of a type described in a previous paper from this laboratory.[9] The apparatus is shown in figure 3, and examples of the results obtained in figure 4. From them it is clear that in a quick expiration through the mouth the "spike" of alveolar air begins to issue between the lips with the first 25 to 50 cc. of air expelled. They show also that for an approximately complete washing out of the dead space, so that the last portion shall consist of practically undiluted alveolar air, an expiration (especially

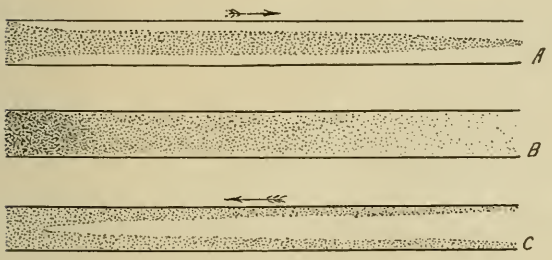

Fig. 1

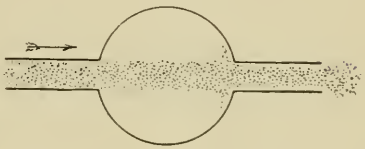

Fig. 2

Fig. 1. (*a*) Shows a "spike" of smoke moving through a glass tube. (*b*) Shows the condition when the current is suddenly stopped and mixing instantaneously occurs. (*c*) Shows clear air drawn in.

Fig. 2. Shows how a column of smoke crosses a bulb with little mixing or sweeping out of the air within it.

if rapid) of at least 400 cc. or three times the volume of the dead space is required. This applies to ordinary quiet breathing. During hyperpnoea the larger dead space necessitates a much greater expiration before an undiluted sample of alveolar air can be obtained.

We have used and compared five methods with minor variations for determining the dead space:

First, or CO_2 *method.* The subject began with the deepest possible expiration with the mouth open, and followed this with an inspiration sufficient in volume to fill the dead space with entirely fresh air. In some cases this breath was held for seven to ten seconds; in others the next act followed immediately. This consisted in the subject making the deepest possible expiration through a rubber tube into a gasometer

or rubber bag. The subject then kept his tongue against the end of the tube until it was closed with a spring clip. A sample of alveolar air was drawn from the tube as in the method of Haldane and Priestley, and its CO_2 percentage (a_1) was determined with a Haldane apparatus. (The little apparatus graduated up to 10 per cent for CO_2 only is most convenient for this purpose.) A sample of the mixed air (m_1) in the gasometer was also analyzed. The volume of the (second) expiration

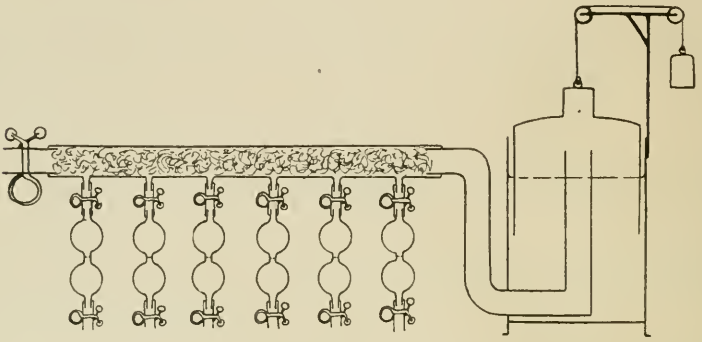

Fig. 3

Fig. 3. Apparatus for analyzing successive portions of an expiration. The volume expired is measured by means of the gasometer. The tube (volume 400 cc. and length 100 cm.) is filled with loosely shredded glass wool or jute. The pipettes hanging from it are filled with $\frac{1}{10}$ normal baryta. Immediately after an expiration the clip at the end of the tube is closed. By opening the clips on the pipettes, exactly one-half (15 cc.) of the baryta is then allowed to run out of each, thus drawing in a sample of air from the tube. The clips are reclosed, and the remaining half of the baryta is thoroughly shaken with this air, and is then drained into a small flask, and tightly stoppered. After complete sedimentation of the $BaCO_3$, a sample (5 cc.) of the supernatant baryta is titrated with $\frac{1}{10}$ normal acid; and, with corrections for the barometric pressure and temperature when the air sample was taken, its CO_2 percentage is calculated.

(E) was read from the gasometer, or by means of a gas meter connected with the rubber bag. The dead space (d) of the apparatus, i.e., the volume of the tube, was determined once for all. It should be as small as convenient. By using a readily collapsible tube, or by filling the tube with water before each experiment, it can be eliminated entirely. Although more exact these arrangements are also more trouble and not necessary.

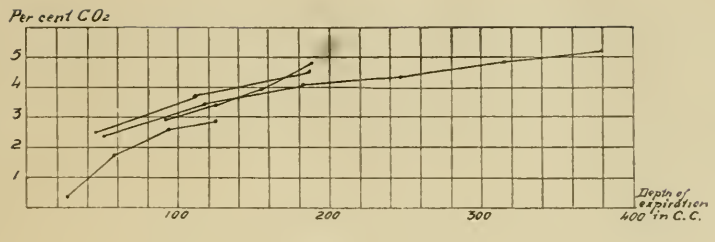

Fig. 4

Fig. 4. Showing the percentages of CO_2 in successive portions of quick expirations in four experiments with the apparatus of figure 3.

The (virtual) dead space (D_1) for CO_2 was calculated from these data by the formula:

$$D_1 = E - E\frac{m_1}{a_1} - d$$

It is not necessary that the alveolar samples should have a normal CO_2 content, as it is not the absolute amount but the relation of this factor to the CO_2 in the mixed air which counts. For instance, in two experiments (on Y. H.) the data and results were:

E	A_1	M_1	d	D_1
3810	3.75	2.90	195	670
3850	6.20	4.70	195	665

The characteristics of the results obtainable with this method are shown by figure 5.

These data and their significance may be summarized as follows: (1) The dead space as determined by this method (without a pause) varies with the extent to which the lungs are inflated. Below the inflation of quiet breathing it may be as small as 60 cc. or less. At the normal level volumes of 150 or 160 cc. are obtained in close agreement with other observers. When the lungs are inflated to an extent corresponding to that of hyperpnoea, the volumes are much larger (up to 600 or 700 cc. or more). But they are the same no matter whether the subject is at rest or exercising. This indicates that the increased dead space observed by Douglas and Haldane under the latter condition is chiefly a mere mechanical stretching, not an active broncho-dilatation.

(2) When inspiration is as far as possible of a costal character, the

values obtained even by fairly great inflation (3100 to 3800 cc.) are only about half as large as when it is of the ordinary diaphragmatic character. This indicates that the expansion of the dead space between ordinary and deep breathing depends to a great extent upon the lengthening of the bronchi and bronchioles with the downward movement of the diaphragm.

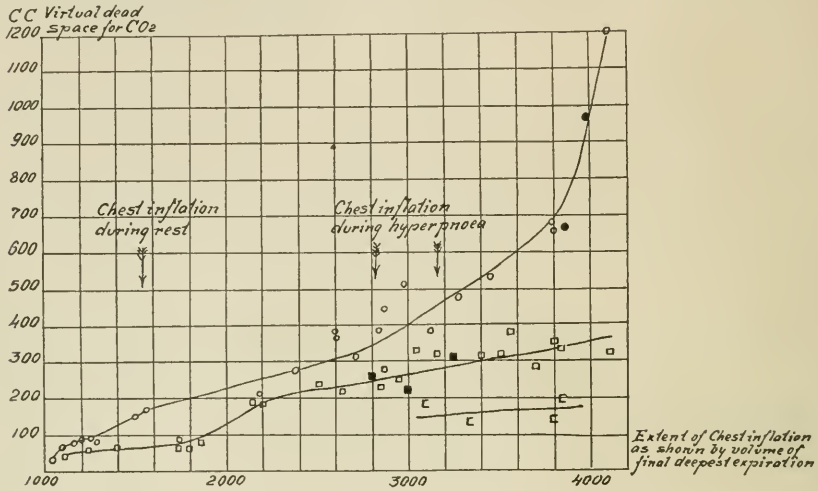

Fig. 5

Fig. 5. A diagram of results obtained by our first method. Ordinates express the (virtual) dead space, and abscissae the extent to which the lungs were expanded above the residuum at deepest expiration. In the subject (Y. H.) during quiet breathing with a tidal volume of 500 cc. inspiration reaches a level of about 1600 cc. as indicated by the arrow at the left, and an active hyperpnoea raises the level to between 2900 and 3600 as indicated by the arrows at the right.

The values for the dead space when the final expiration was made immediately after the inspiration are indicated by the little circles (o)., and upper line.

The values obtained when the inspiration was held for seven to ten seconds are expressed by the little squares (□) and the middle line.

The influence of making the inspiration and pause so far as possible with the costal mechanism without contraction of the diaphragm is shown by the incomplete squares (⊏) and the lower line.

All of the foregoing observations were made while the subject was at rest, sitting on a stool in a room of comfortable temperature. The solid circles and squares (● ■) on the contrary express observations by identical procedures, but taken while the subject was hyperpnoeic from vigorous exercise on a stationary bicycle. As they correspond closely with the observations during rest, they show that the increased dead space during exercise is not due to active bronchodilatation, but to passive distension incident to deep inspirations.

(3) When the inspired air is held for seven to ten seconds before the final expiration is made, the values obtained for the virtual dead space are at all levels of chest inflation considerably smaller (less by a third to one-half) than when no pause is made. This is probably due to diffusion of CO_2 in appreciable amounts through the mucosa of the mouth, trachea, bronchi, etc. (A study of the diffusion of CO_2 and oxygen in the mouth is now under way in this laboratory, has afforded confirmation of this view, and will be reported in a later paper.)

Second, or oxygen method. The procedures were the same as in our first method except that the gas samples were analyzed for oxygen and the percentages found (m_2 and a_2) were subtracted from the oxygen percentage of the inspired air. Thus the dead space for oxygen (D_2) was found by the formula

$$D_2 = E - E\frac{20.93 - m_2}{20.93 - a_2} - d$$

It is noteworthy that when both this and the CO_2 method were carried through on the same breath *the (virtual) dead space for CO_2 was always smaller than that for oxygen.* The difference between them was greater (owing to the fact that D_1 was reduced much more than D_2) when the breath was held for several seconds than was the case when no pause was made. For example we found (on J. L. W.):

	E	D_1	D_2	$D_1 : D_2$
With pause.....	720	33	78	42:100
With pause.....	1450	66	192	34:100
With pause.....	3430	569	1020	55:100
Without pause.....	(800)	130	154	84:100
Without pause.....	(1450)	156	164	95:100
Without pause.....	(3000)	563	809	69:100

The last three experiments here instanced were really made with our fourth and fifth methods which give the average dead space for a series of breaths. But they suffice to show that the difference between D_1 and D_2 is much less without, than it is with, a pause. From this fact as from the evidence of the first method above discussed, it is clear that *there is a very considerable diffusion of CO_2 from the walls of the respiratory passages.* The oxygen exchange on the other hand between the air in these tubes and the blood in their walls is comparatively small. It is probable that the great part of the blood affected by this

diffusion is not in the pulmonary, but in the systemic circulation and flows from the bronchial veins to the right heart.*

This bronchial CO_2 diffusion affords an explanation of the fact that the respiratory quotient calculated from alveolar analyses is always lower than that found from the total expired air. Indeed this explanation was pointed out to one of us some years ago by Dr. J. S. Haldane,[10] and it was his forecast which led us to look for a smaller (virtual) dead space for CO_2 than for oxygen.

Third, or hydrogen method. This is the method of Siebeck as employed by Krogh and Lindhard. In our use of it we made a pause of some six to eight seconds before the final expiration. We also varied the depth of the inspiration, the point which, as already explained, Krogh and Lindhard overlooked. In several experiments we determined on a single breath the dead space, both for oxygen and for hydrogen. The formula for the method, in which H is the percentage of hydrogen in the gas inspired, m_3 and a_3 are the percentages of hydrogen in the mixed and alveolar samples, and the other terms, as in previous formulae, is

$$D_3 = E - E\frac{H - m_3}{H - a_3} - d$$

Our results with this method are given in the fourth column of the table of comparative results.

The dead space for hydrogen is, in general, of approximately the same size as that for oxygen. Like that both for oxygen and for CO_2, it varies with the extent to which the lungs are inflated. These observations do not, however, invalidate the demonstration of Krogh and Lindhard that with breaths of the same size the dead space is the same during exercise as during rest.

Fourth and fifth, or Douglas bag methods. The fourth method which we used was identical with that of Douglas and Haldane. The total expired air for a certain length of time was caught in a Douglas bag and measured. This volume was divided by the number of breaths made in the period to find the mean volume of expiration (e). A sample was analyzed for CO_2, and a separate determination of the subject's alveolar air was made. The formula was the same as that for our first method, except that the mean tidal air (e) replaces (E)

*Since this paper was written, correspondence with Dr. Haldane has changed our opinion on this point. The greater part of this blood must go into the pulmonary veins and to the left heart. See note at the end of this paper.

the deepest possible expiration. The extent of the lung dilatation (E) when needed, was determined separately with a small graduated spirometer.

The fifth method was the same as the fourth except that oxygen analyses also were made on the expired and alveolar air. The formula was the same as for our second method with (e) substituted for (E).

With these methods the dead space for oxygen was always larger than that for CO_2. But as there was no pause between inspiration and expiration the differences were not nearly so great as between the results of the first and second methods with a pause.

The effects of exercise (fast walking) were found, in agreement with the observations of Douglas and Haldane, to include a considerable increase in the dead space. But this was not to any considerable extent assignable to active broncho-dilatation, for it was of practically the same amount as that obtained by the other methods with equal chest inflation while the subjects were at rest. Furthermore, with the bag methods when the subject sat perfectly still and voluntarily made deep, but slow breaths, the dead space worked out to a volume as great as, or greater than, that during the hyperpnoea of exercise. For example, the dead space for CO_2 and for oxygen (D_4 and D_5) were found (on J. L. W.) to be:

	TIDAL AIR	D_3	D_4
At rest, shallow breathing with constricted chest	180	130	154
At rest, natural breathing	403	189	198
Fast walking and natural hyperpnoea	1373	407	650
At rest, deep slow breathing	1384	563	809
At rest, deep slow breathing	2116	917	1237

Comparative results. In the table of comparative results are shown data for a single subject by all five methods. The first column indicates the extent to which the lungs were dilated above deepest expiration. In this subject when seated and breathing quietly the tidal air amounted to 400 or 450 cc. In ordinary inspiration his lungs were dilated to 1400 or 1500 cc. above deepest expiration. The "vital capacity" was 3800 or 3900 cc. The data of the fourth and fifth methods are inserted at places in the table corresponding to the extent to which the lungs were expanded at inspiration.

Table of comparative results of determinations of the volume of the dead space of a single subject (J. L. W.) by five methods (D_1 to D_5) arranged according to the extent to which the lungs were dilated (E)

E	D_1	D_2	D_3	D_4	D_5	
			cc.			
720	33	78				
725			138			Below level of normal breathing, i.e., with contracted chest.
860	58					
				130	154	
				154	163	
				132	154	
1450	66	192				
				189	198	At level of normal breathing.
1480			102			
1500	191	296				
1650	82	245				
1710	86	186				
1860		370	267			
2430			139			
2540	156					
2710			234			
2770	290					
				407	650	At level of hyperpnoea of vigorous exercise.
				563	809	
3220		1116	1105			
3400			653			
3430	569	1020				
3450		847	725			
3460	289	620				
3550	406					
3580			928			
3620	796	1212				
				917	1237	

The table clearly demonstrates by all methods the passive dilatation of the dead space with chest expansion. It shows the diffusion of CO_2 from the walls of the dead space by the facts that the figures for D_4 are somewhat smaller, and those for D_1 (with a pause) are very much smaller, than those obtained by the hydrogen (D_3) and the two oxygen methods (D_2 and D_5).

There is also to be noted a contrast between the first three methods (on single breaths) and the fourth and fifth (with the Douglas bag) in that, while the latter increase fairly uniformly, the former are quite

irregular. These discrepancies are not due to analytical errors which can scarcely exceed 10 per cent (except possibly in the third, or hydrogen method, where they may reach 20 per cent), nor to variations in the final measured expiration which should not err more than 100 or 200 cc. in totals of from 1000 to 4000 cc. They are due to the fact that, while the fourth and fifth methods give the mean dead space for several minutes, the first three methods determine it at a single instant. This suggests that the dead space is continually undergoing active variations which are recorded by our first three methods and averaged by the last two.

Rhythmic variations in the dead space. When one makes a series of determinations at regular intervals by any one of our first three methods on a single subject under conditions and at a chest expansion as nearly uniform as possible, it seldom happens that any two successive results agree to within even the extreme errors of the method. This fact which we have verified repeatedly puzzled us even after many months had been spent in unravelling the influence of chest expansion on the dead space. The suggestion was made to us by Dr. A. L. Prince of this laboratory that there might be variations of tonus in the non-striated muscle fibers of the bronchi similar to those in other organs containing such tissue. A survey of the literature shows that Einthoven[11] many years ago observed slight rhythmic variations of bronchial tonus in dogs, although more recent investigators seem not to have noticed them.

Accordingly we carried through by our first method two series of observations (on J. L. W.) in which the dead space was determined every three minutes for more than an hour.* The results are shown in figures 6 and 7. In figure 6 are to be seen rhythmic variations of a periodicity of about 7.5 minutes, large waves amounting to thirty or forty per cent of the whole volume of the dead space alternating with waves only half as large. In figure 7 the results obtained on the same subject the next day exhibit equally marked variations, but of slower and less clearly marked periodicity.

We may fairly conclude from these experiments, supported by a mass of observations too voluminous for detailed publication, that *the respiratory dead space, like other cavities having non-striated muscle fibers in their walls, is subject to considerable active variations of a more or less rhythmic character.*

*We are indebted to Dr. Prince for assistance in carrying out the necessary rapid succession of analyses.

Reflex influences of close and fresh air on bronchial tonus. One of us (Y. H.) is very susceptible to the ill effects of a close and crowded room. The nasal mucosa becomes congested almost to the occlusion of the nostrils, and asthmatic sensations also develop. On going out of doors, or feeling a cool breeze on the face, there occur a reflex constriction of the nasal blood vessels, and an ease of breathing, after a few deep breaths, suggestive of an active reflex change from constriction to dilatation of the bronchi. The stimulus seems to be the cool sensation from the face. The effect is too rapid to depend on body temperature. Sensations of "stuffiness" and obstructed breathing have been noticed also in a crowded railroad car when there was no perceptible perspira-

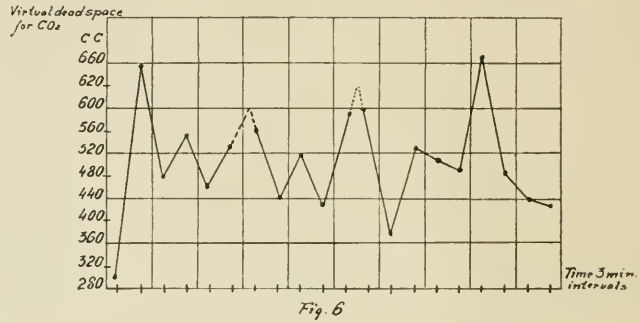

Fig. 6

Fig. 6. A series of dead space determinations (averaging 500 cc.) at intervals of three minutes for over an hour made by our first method on J. L. W. The chest expansion was as nearly uniform as possible: averaging 3300 cc. for E. Active variations in the volume of the dead space are here seen to occur rhythmically, large waves alternating with small at intervals of about seven and one-half minutes.

tion, no impression of being too warm, and occasionally even when it was rather too cold for comfort. We have noticed also that in a Turkish bath, persons who are unaccustomed to it, or who do not perspire readily, may experience obstructed breathing, nausea and faintness, while the practiced bather breathes quite easily and feels exhilarated. The same holds true, however, in a steam room (Russian bath) with an atmosphere at 45° C., and saturated with moisture. Under the latter conditions body temperature must rise practically equally whether one perspires or not. The obstructed breathing, if it is a broncho-constriction, together with the nausea and faintness, appear to us, there-

fore, to be rather due to reflexes from the skin than to the central effects of hyperthermia.[12]

Early in these investigations there were carried out on Mr. Edmund Andrews, then a student in this laboratory, a series of observations which indicated *that in a "close" room with a distinct feeling of stuffiness the dead space is abnormally small; that standing in an agreeably cool breeze from an open window for even a few seconds induces an enlargement; that a distinct chill on the contrary induces in one coming from a close room, not an increase, but on the contrary a further decrease in the volume of the dead space—in one case the subject "caught cold;" and that a warm room with no sensation of stuffiness but free perspiration tends to cause enlargement of the dead space.* The methods employed for these observations were crude (the apparatus shown in figure 3 was used) and

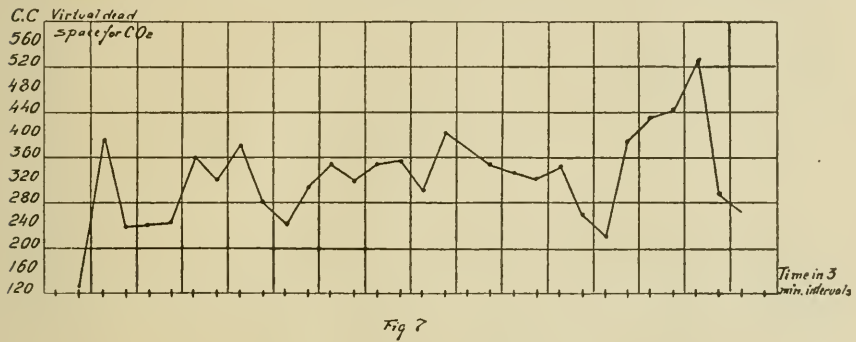

Fig. 7. A series of observations similar to those in figure 6 on the same subject the next day. The rhythm is much less regular. The mean (330 cc.) of these determinations is less than before because E was smaller, averaging 2650 cc.

we had not then puzzled out the relation of the volume of the dead space to chest inflation. The observations are, however, worthy of record as a starting point for future investigations.

Using our second method and expirations as nearly equal as possible (3260 and 3140 cc.) we found the dead space (in J. L. W.) in a close room to be 645 cc. and a few minutes later out of doors—air cool and dry, and sky clear—a volume of 816. Owing, however, to our demonstration of the type of variations discussed in the preceding section, it will require many additional observations to settle the point as to whether this really indicates broncho-dilatation.

We have, however, carried out several experiments on the influence upon the dead space of steam, hot air, and cold shower baths. The

results are rather discordant, although they suggest that when one begins taking such baths the hot rooms cause broncho-constriction, and the cold shower a dilatation, but that after a little practice reactions of the opposite type occur. Our initial experiments on the effects of the hot room (65° C. with dry bulb thermometer and 37° with wet bulb), steam room (44° C. with both wet and dry bulb thermometers), and cold shower (16°) of a Turkish bath gave the following results for the oxygen dead space (D_2) at nearly uniform chest inflation (E):

SUBJECT	Y. H.	J. L. W.
E_1 or amount of lung inflation	3150–3320 cc.	3340–3650 cc.
D_2 in cool well ventilated room	230	1346
D_2 after fifteen minutes in steam room	104	218
D_2 after fifteen minutes more in hot room		
D_2 after cold shower		634
D_2 after dressing cool room	338	1124

A few days later, the following results were obtained.

SUBJECT	Y. H.	J. L. W.
E_1 or amount of lung inflation	3650–4100 cc.	3550–3800 cc.
D_2 in cool dressing room	300	505
D_2 after ten minutes in steam room	385	777
D_2 after ten minutes more in hot room	752	1288
D_2 after cold shower	234	557
D_2 after dressing in cool room	360	1270

One experiment on this topic was made also with the Douglas bag methods. It showed that (in J. L. W.) after ten minutes in the steam room and similarly in dry heat the (virtual) dead spaces for CO_2 and for oxygen were 200 cc. and 396 cc. respectively, as compared with 189 cc. and 198 cc. when the subject was sitting in a room of ordinary temperature. But the volume of air breathed per minute, and the tidal volume were about twice as great in the hot rooms as in the cool, so that the chest inflation (which we failed to measure) was probably enough to balance to an unknown extent the apparent broncho-dilatation. This incomplete experiment, however, yielded one new observation: it indicated that *in great heat the exhalation of CO_2 from the walls of the dead space is enormously increased. This may well be due to an active hyperaemia.* This exhalation is shown in the fact that although

the subject was breathing twenty times a minute the virtual dead space for CO_2 (D_4) was only half of that for oxygen (D_5). In agreement with the observations of Haldane,[13] the alveolar CO_2 was considerably lowered. The respiratory quotient for the mixed expired air was 0.928, while the quotient calculated from the alveolar air was only 0.717: an extraordinary difference.

The dead space in asthma. Some observations on asthmatic subjects (by F. P. C. at the Tulane Medical School in New Orleans) show dead spaces (D_1) smaller than in normal subjects although not to the extent expected.[14] It is very probable, however, that their chests were dilated much above the normal level and that they failed to make expirations of normal depth in the tests. When these measurements were made, we were still unaware of the influence of chest expansion on the dead space. Assuming their chests to have been expanded to the extent of 1000 or 1500 cc. above the normal their dead spaces were only about one-third of that of normal subjects at such chest expansions. *It appears probable that in asthmatics the abnormally expanded condition in which the chest is held affords a passive stretching of the bronchi and bronchioles which partially compensates for the active bronchoconstriction.*

CONCLUSIONS

From a consideration of the axial flow of gases through tubes, and from determinations of the CO_2 content of successive fractions of the expired air it is found that in man some alveolar air (i.e., CO_2 in increasing amounts), begins to issue from the nose and mouth even in the first 50 cc. of an ordinary expiration. A tidal volume even much smaller than the volume of the dead space may thus afford a very considerable gaseous exchange, as in animals during heat polypnoea. With the ordinary expansion of the chest during quiet breathing an expiration of at least 400 cc. is necessary to effect an even approximately complete washing out of a dead space of 150 cc. During hyperpnoea a much larger expiration is necessary before a sample of pure alveolar air is obtainable.

Five methods of determining the dead space on man have been employed with generally concordant results and some significant differences. The results show that the dead space expands and contracts passively with the movements of the thoracic walls and lungs. At the level of ordinary breathing the dead space is about 150 cc. (as practically all previous observers have found). At shallower levels, it is, however, considerably less; with deeper breathing it is much

more (400 to 600 cc.); and with the deepest breaths the (virtual) dead space may exceed a liter in volume. It is much more affected by diaphragmatic than by costal movements.

The dead space is found to be of practically the same volume during rest and exercise, providing that the determinations are made at equal extents of chest inflation. This fact indicates that the enlargement of the dead space during hyperpnoea is essentially a passive stretching and not an active broncho-dilatation.

The dead space for oxygen is always larger than that for CO_2. This is shown to be due to the diffusion of CO_2 in considerable amounts from the walls of the mouth, trachea, bronchi, etc.

The dead space, even at a uniform extent of chest inflation, is continually undergoing active variations in volume. At times these variations exhibit a distinct rhythm of a period of several minutes. They may amount to as much as 30 per cent of the mean volume of the dead space in quiet breathing.

Some facts are reported which suggest that in a "close and stuffy" room the bronchi, etc., are constricted, and that a distinct chill constricts them further, while pleasantly fresh cool air, on the contrary, induces broncho-dilatation. Experiments in a Turkish bath have not, however, afforded concordant results, except that they have shown that during profuse perspiration and cutaneous hyperaemia there is a greatly augmented diffusion of CO_2 from the walls of the dead space. This doubtless indicates a hyperaemia of the respiratory passages. As a part of the CO_2 given off in the passages is inspired into the alveoli before being expired, and as the alveolar respiratory quotient may be only a little above 0.7 when the quotient of the mixed expired air is considerably above 0.9, it appears that under such conditions as much as one-half of the total CO_2 exhaled by the subject may come from the dead space.

In asthmatics, the chronic dilatation of the chest stretches the pulmonary passages passively, and thus tends to compensate to some extent for their active contraction.

Note: After our investigations were completed and this paper was ready for publication, one of us (Y. H.) wrote to Dr. J. S. Haldane of the results obtained. By return mail, Dr. Haldane replied that he had been at work on the same topic and had obtained practically identical results as regards the passive expansion of the dead space.

A few days later he sent to us the manuscript of his paper with the suggestion that it should either be combined with ours or pub-

lished simultaneously. As publication of the two papers uncombined appears to us to be the most effective method of carrying conviction to the minds of others, and as each paper has some special aspects, Dr. Haldane's paper (which he has curtailed and modified after reading our paper) appears elsewhere in this number of this Journal. It is certainly a rare event that investigators, working entirely independently on opposite sides of the Atlantic, reach so nearly the same conclusions on a topic which has been in a state of confusion as long as has that of the respiratory dead space. The only important point of difference, between Dr. Haldane and ourselves was as to whether the greater part of the blood, which has lost CO_2 directly to the dead space, passes to the right heart, as we supposed, or to the left heart, as Dr. Haldane suggests. In the latter case the large amounts of CO_2 given off to the respiratory passages during hyperpnoea, and especially during heat hyperpnoea and bronchial hyperaemia (see our experiments in the Turkish bath) may prove to be of great importance. After reading the papers of Miller[15] to which Dr. Haldane refers there is no doubt in our minds that Dr. Haldane is correct in considering that the passive stretching of the dead space occurs principally in the atria, and that the greater part of the blood from the dead space flows in to the pulmonary veins and left heart.

[1] For literature cf. Siebeck: Skandinavisches Archiv für Physiologie, 1911, xxv, p. 81.

[2] Loewy: Pflüger's Archiv, 1891, lviii, p. 416.

[3] Douglas and Haldane: Journal of Physiology, 1912, xlv, p. 235.

[4] Siebeck: Loc. cit.

[5] Krogh and Lindhard: Journal of Physiology, 1913, xlviii, p. 30.

[6] Cf. Campbell, Douglas and Hobson: Journal of Physiology, 1914, xlviii, p. 303.

[7] Similar observations have been made but misinterpreted by Carter.: Journal of Experimental Medicine, 1914, xx, p. 81.

[8] For literature see Jackson, D. E.: Journal of Pharmacology and Experimental Therapeutics, 1914, v, p. 479.

[9] Henderson and Russell: American Journal of Physiology, 1911, xxix, p. 441.

[10] Pike's Peak Expedition: Phil. Trans. B. cciii, p. 231.

[11] Einthoven: W. Pflüger's Archiv. f. d. gesammte Physiologie, 1892, li, p. 415.

[12] Cf. Henderson: The unknown factors in the ill effects of bad ventilation. Transactions of Fifteenth International Congress on Hygiene and Demography, 1913, ii, p. 622.

[13] Haldane: Journal of Hygiene, 1905, v. p. 494.

[14] Hoover, C. F. and Taylor, L. have reported similar observations but have interpreted them differently: Archives of Internal Medicine, 1915, xv, p. 1.

[15] Miller, W. S.: Journal of Morphology, 1893, viii, p. 165; and Anatomomische Anzieger, 1906, xxviii, p. 433.

THE VARIATIONS IN THE EFFECTIVE DEAD SPACE IN BREATHING

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From Cherwell Laboratory, Oxford

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In the foregoing paper by Messrs. Yandell Henderson, Chillingworth and Whitney, clear evidence is brought forward that the increase in the respiratory dead space during hyperpnoea, as noted by Douglas and myself during muscular work, and by Campbell, Douglas and Hobson for hyperpnoea caused by CO_2,[1] is due, not to active dilatation of the bronchi, as we believed, but to mechanical stretching of the lungs. I had meanwhile reached the same general conclusions, and it is unnecessary for me to repeat what my American colleagues have so admirably expressed; but as my methods differed in certain respects from theirs, and in one or two points I have been led to a different interpretation of the data, it seems worth while to put my experiments on record along with theirs.

Douglas and I obtained our results for the dead space with the existing natural breathing, deep during hyperpnoea, and comparatively shallow during rest. I had been led to suspect that the apparent divergence between our results and those of Krogh and Lindhard depended on the depth of breathing, and in order to determine the influence of varying the depth *per se*, without any hyperpnoea, I made use of the fact, discovered by Priestley and myself, that the frequency of breathing may be varied within wide limits, without altering the alveolar CO_2 percentage, provided that the depth of breathing is allowed to regulate itself naturally, with no forcing or holding back. Thus by varying the frequency one can greatly vary the depth, without altering the mean alveolar CO_2 percentage, and without true hyperpnoea being present. I have verified this on myself within wider limits than in our original experiments, in groups of experiments, the experiments in each group succeeding one another at as short intervals as possible, but the different groups being on different days.

[1] Journal of Physiology, xlv, p. 235, 1912, and xlviii, p. 303, 1914.

It was easy enough to reduce the frequency to three breaths a minute without any discomfort, provided the inspirations and expirations were sufficiently slow and regular. The results obtained were as follows:

Alveolar CO_2 percentage

FREQUENCY OF RESPIRATIONS PER MINUTE	END OF INSPIRATION	END OF EXPIRATION	MEAN
30	5.66	5.70	5.67
4	5.24	6.09	5.66
24	5.48	5.49	5.48
6	5.40	5.73	5.56
36	5.63	5.73	5.68
4	5.11	6.34	5.72
3	5.19	6.24	5.71
60	6.17	6.16	6.16

The constancy of the alveolar CO_2 percentages with frequencies of from three to thirty-six breaths is very striking. The cause of the paradoxical rise in alveolar CO_2 percentage when the frequency was increased to sixty will be discussed later.

The effective dead space was now determined by our method with varying frequencies of breathing, and consequent variations of depth, the expired air being collected over a period of three minutes, and the rate of breathing having been accurately adjusted by a clock for at least two minutes before the collection of samples was begun. Inspiration and expiration were timed to be of about equal duration and with no pause between them. For reasons which will appear below, the results, which are given in the following table, are stated somewhat fully.

This table shows clearly that in spite of the absence of hyperpnoea the effective dead space increases enormously with increased depth of breathing, the increase in dead space bearing a rough proportion to the increase in depth. This fact explains the apparent divergence between our own results and those of Krogh and Lindhard, since the latter observers made their determinations with a constant and relatively small depth of breathing.

The data for oxygen bring out a further point. It will be noticed that the differences between the oxygen percentages at the end of inspiration and end of expiration are much greater than the differences in the CO_2 percentages: also that the respiratory quotient as calculated

FREQUENCY OF RESPIRATIONS PER MINUTE	MEAN DEPTH OF EXPIRATIONS AT 37° SATURATED IN CC.	EXPIRED AIR			ALVEOLAR CO_2 PERCENTAGE			ALVEOLAR O_2 PERCENTAGE			ALVEOLAR RESPIRATORY QUOTIENT	EFFECTIVE DEAD SPACE MINUS THAT OF MOUTHPIECE, CC.	
		CO_2%	O_2%	Respiratory quotient	End of inspiration	End of expiration	Mean	End of inspiration	End of expiration	Mean		Calculated from CO_2	Calculated from O_2
3	2984	4.29	16.07	0.848	5.41	6.04	5.72	14.56	12.84	13.70	0.745	683	920
4	2438	4.56	16.91	0.875	5.24	6.09	5.66	14.98	13.07	14.02	0.814	467	619
6	1413	4.24	16.47	0.905	5.37	5.70	5.53	14.51	14.25	14.38	0.803	272	392
18.5	683	3.31	17.27	0.871	5.53	5.75	5.64					224	
17.0	650	3.59	17.01	0.887	5.63	5.79	5.71	13.95	13.74	13.84	0.762	171	223
17.7	643	3.58	16.98	0.888	5.50	5.59	5.55	13.44	13.99	13.72	0.721	161	231
24	410	3.22	17.15	0.780	5.45	5.46	5.45	13.88	13.42	13.65	0.692	111	136
60	357	1.89	18.75	0.820	5.87	6.06	5.96	13.15	12.85	13.00	0.703	185	199

from the alveolar samples is lower than the true quotient as calculated from the composition of the expired air. To the latter point and its probable explanation attention had already been called by Douglas, Henderson, Schneider and myself in our account of the Pike's Peak Expedition.[2] It follows that, as shown in the table, the effective dead space calculated from the oxygen percentages is greater than that calculated from the CO_2 percentages, my results in this respect being entirely confirmatory of those given in the previous paper.

It will also be seen that in the three experiments with normal breathing, and a depth of breathing of about 650 cc., the dead spaces found differed considerably, thus also confirming the conclusions of the previous paper.

It seemed probable to Douglas and myself that the increase in the dead space during hyperpnoea is due to general relaxation of the bronchial muscular coat, so that air can pass more easily. As, however, the increase occurs without any hyperpnoea, this view becomes untenable: the more so as Professor Dixon informs me that he had meanwhile found in direct experiments on animals that no broncho-dilatation occurs on administering air containing CO_2.[3] It is thus necessary to seek for another explanation.

Considering that the walls of the bronchi are very thick relatively to the walls of the freely distensible air spaces of the surrounding lung-tissue, it does not seem probable that any considerable dilatation of the bronchi can be brought about by mere mechanical distension of the lungs in deep breathing. The position at which the increase of dead space occurs must therefore, I think, be sought beyond the terminal bronchioles. The manner in which a terminal bronchiole in the mammalian lung breaks up was carefully worked out by reconstruction and other methods by W. S. Miller, and is clearly described and figured in his paper.[4] Miller's work seems to furnish the key to the interpretation of the increased dead space. Each terminal bronchus (see fig. 7 and 8 of Miller's paper), ends in several openings or "vestibules," each of which leads into an air-cavity or "atrium," lined

[2] Phil. Trans., B, cciii, p. 221.

[3] The method he used was that employed in the experiments of himself and Ransom on broncho-dilator nerves (Journal of Physiology, xlv, p. 413, 1912). Einthoven observed broncho-constriction under the influence of CO_2 with the vagi intact, and no effect after vagus section. Einthoven, W: Pflüger's Archiv f. d. gesammte Physiologie, 1892, li, pp. 411 and 423.

[4] W. S. Miller, Journal of Morphology, viii, 1893, p. 165.

by alveoli. From each atrium several openings lead onwards into "air-sacs," which are main cavities of which the walls are constituted by alveoli or air-cells. By far the greater number of the lung alveoli belong to the air-sac system, but a very appreciable number belong to the atria, and the latter act partly as air-passages to the air-sacs, and partly perform the same respiratory functions as the air-sacs themselves. The walls of the atria have the same general structure as those of the air-sacs, and must be just as free to expand when air enters the lungs.

It is evident that the atria must have a far greater supply of fresh air than the groups of air-sacs beyond them, since all the fresh air supplied to the air-sacs passes through the atria, and at the end of an inspiration they will be left full of relatively pure air. They will therefore contribute to the "effective" or "virtual" dead space due to the bronchi and upper respiratory passages; and as they will expand freely with a deep inspiration, and be washed out more thoroughly, the dead space will increase with a deep inspiration.

If the lungs as a whole are over-ventilated by temporary forced breathing, the respiratory quotient, as calculated from the composition of the expired air, is extremely high, since over-ventilation extracts much extra CO_2 from the blood, but cannot impart appreciably more oxygen to it. As, however, the atria are, as it were, constantly over-ventilated, the part of the expired air coming from them will have a high respiratory quotient. The air from the air-sacs must therefore have a lower quotient, so that the mixed expired air, coming from atria and air-sacs, has a quotient representing that for the body as a whole. As, moreover, the pulmonary blood passes partly through the atria, though mainly through the air-sacs, the partial pressure of CO_2 in the mixed arterial blood will be slightly lower than that of the blood from the air-sacs, though higher than that of the blood from the atria. This conception explains the fact, noted above, that with very shallow and frequent breathing the excess of CO_2 and deficiency of oxygen in the air-sac air increases.

It is doubtless true that the respiratory exchange of the bronchi and upper air-passages must make some contribution to the total exchange represented in the expired air; but considering the thickness of the bronchial epithelium and the very small mass of the whole mucous membrane lining the bronchi, etc., this contribution must be very small: whereas the extra CO_2 represented by the difference in respiratory quotient between alveolar and expired air represents about 12 per cent of the total CO_2 given off by the body. Moreover it is only during ex-

piration that the bronchial mucous membrane can contribute towards raising the respiratory quotient above that of the alveolar air, since any CO_2 coming off during inspiration is carried down to the alveoli. It seems, therefore, that the respiratory exchange of the bronchial mucous membrane contributes hardly anything to the difference in respiratory quotient between alveolar and mixed expired air. In this conclusion I was confirmed by finding that the respiratory quotient of the first part of the expired air is not strikingly different from that of later parts. The first part would be expected to have a very high respiratory quotient if the respiratory exchange of the bronchi were responsible for the respiratory quotient of the expired air being so much higher than that of the alveolar air.

Priestley and I found that when, during normal breathing, air is expelled sharply from the lungs, the partial pressure of CO_2 in the expired air is constant after a certain amount of air has been expelled, and we inferred that the air of constant CO_2 pressure is alveolar air. It now appears that the air in question is alveolar air from the "air-sacs" of Miller's nomenclature, and that the air from the alveoli of his "atria" is of a different and more variable composition. I have made a few experiments in order to test more definitely than in our original experiments the depth of expiration needed in order to obtain air of constant composition. A rubber bag, of which the capacity when inflated could be varied at will by the adjustment of a large wooden clamp, was attached to the far end of the piece of tubing used for obtaining samples of alveolar air. As the inflation of this bag stopped the expiration, samples of the air leaving the mouth after any desired depth of expiration could be obtained. With ordinary resting breathing (about 18 breaths per minute in my case) the following results were obtained in successive trials on the same day.

DEPTH OF EXPIRATION	PERCENTAGE OF CO_2 IN AIR ISSUING FROM MOUTH	DEPTH OF EXPIRATION	PERCENTAGE OF CO_2 IN AIR ISSUING FROM MOUTH
1350	5.51	650	5.27
190	3.03	650	5.32
335	4.37	650	5.18
510	5.17	1350	5.57
510	4.91	950	5.49
1350	5.39	950	5.53
1350	5.37	1350	5.44
650	4.95	1350	5.63
650	5.23		

If we average this series the results are:

DEPTH OF EXPIRATION	CO_2 PERCENTAGE IN AIR ISSUING FROM MOUTH
190	3.03
335	4.37
510	5.04
650	5.19
950	5.51
1350	5.48

In ordinary determinations of the alveolar CO_2 percentage about 1350 cc. was, so far as I could judge, about the depth of expiration usually employed in my own case. By somewhat forcing the expiration about 1750 cc. could, however, be expired without causing undue delay. A further series (on a different day) was therefore made, to see if any change could be detected in the deepest portions of the expired air. The results were that in six successive determinations the mean percentage of CO_2 was 5.39 with an expiration of 900 cc., and 5.36 with an expiration of 1750 cc. Hence the deeper part of the expiration contained no more CO_2 than the middle part.

A few experiments were also made in order to see what depth of expiration is needed in order to reach a constant CO_2 percentage in a sample taken at the end of inspiration when the breathing was deep. The mean results were as follows, with breathing at a frequency of four per minute, and a depth of about 2000 cc. at 12°.

DEPTH OF EXPIRATION IN CC. AT 12°		CO_2 PERCENTAGE IN AIR ISSUING FROM THE MOUTH IN A SHARP EXPIRATION AT THE END OF INSPIRATION
First series	460	4.74
	910	5.11
	2550	5.34
Second series	1340	5.10
	2550	5.15

It appears from these results that a depth of expiration of about 1500 cc. would be needed to obtain a sample of undiluted alveolar air

at the end of an inspiration of 2000 cc. With still deeper breathing the depth of expiration needed would doubtless be greater.

If some of the current descriptions of the manner in which the terminal bronchioles are connected with the alveoli were correct it would be hard to offer any explanation of why the composition of the expired air becomes constant after a certain depth of expiration: for according to these descriptions the further away an alveolus is from the terminal bronchus the less fresh air will it receive. Miller's investigations have made it possible to explain the actual facts, including the increase of the virtual dead space with deep breathing.

With regard to methods used for determining the dead space, it seems worthy of remark that the "effective" or "virtual" dead space is a physiological, and not an anatomical conception. The magnitude of this space depends on the physiological efficiency of the respiratory surfaces in relation to the supply of venous blood and fresh air. It therefore seems wrong in principle to use the method of hydrogen inhalation for the purpose of estimating the effective dead space. It is also evident that the varying magnitude of the effective dead space with different depths and types of breathing, and the differences of the dead spaces calculated for oxygen and CO_2, make the calculation of the composition of the alveolar air from that of the expired air a very uncertain matter. In this connection I may perhaps put on record that my own experiments have confirmed the observation of Messrs. Henderson, Chillingworth and Whitney that a pause at the end of inspiration greatly reduces the effective dead space, as would be expected. Increase of depth of breathing must tend to increase the effective dead space, since the atria are more expanded, but increase of frequency must have the same effect, since the air remains for a shorter time in the atria. In very shallow and frequent breathing these two factors appear to counterbalance one another, as seen in the results obtained with a frequency of 60 per minute.

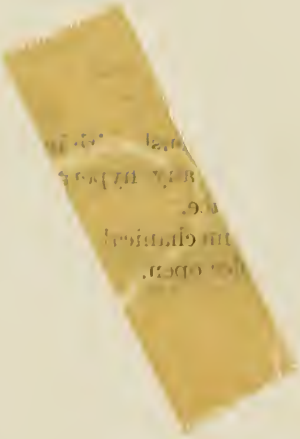
SUMMARY

1. The effective dead space increases enormously with increased depth of breathing, apart from the existence of any hyperpnoea or other causes which might also affect the dead space.

2. This increase is apparently due to mechanical distention of the "atria" into which the terminal bronchioles open.

3. The dead space for oxygen is greater than for CO_2.

4. Estimates of the composition of alveolar air from the composition of the mixed expired air on the assumption of a constant dead space are fallacious.



CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXIV. The Tonus and Hunger Contractions of the Stomach of the New-Born

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The gastric hunger mechanism is probably inherited. At any rate, the frequency and duration of the periods of gastric hunger contractions are related to the feeding habits of the individuals or the species only in so far as the feeding time and the food quantity are factors in the time required for emptying of the stomach, and hence for the appearance of the hunger contractions.[1] On the other hand, the hunger mechanism determines to a certain extent the feeding habit. Animals and children probably eat as soon as the stomach is nearly empty, if food is at hand, and the greater frequency of the gastric hunger periods in the young, as shown by Patterson for the dog,[2] is probably related to the more continuous feeding on the part of the young animal.

We have now made observations on a number of new-born infants, and on two pups, born 8–10 days before term, with results showing that the empty stomach at birth and in the prematurely born exhibits the typical periods of tonus and hunger contractions of the adult,[3] the only difference between infant and adult being the greater frequency and relatively greater vigor of these periods in the young. In the case of the two pups, and in some of the infants, the observations were made before their first nursing. It is thus clear that in the normal mammal the gastric hunger mechanism is completed, physiologically, and is probably active some time before birth.

[1] Carlson: This Journal, 1914, xxxiv, p. 169.
[2] Patterson: This Journal, xxxiii, p. 423.
[3] Carlson: This Journal, 1912, xxxi, pp. 151, 175.

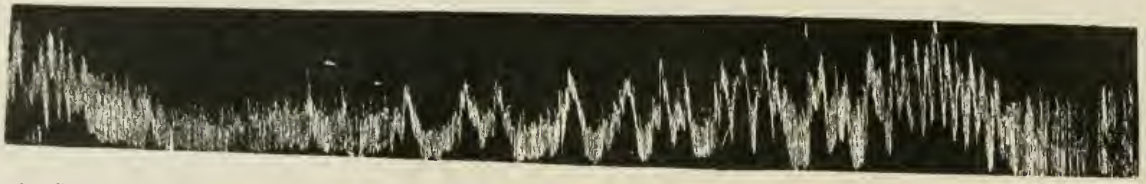

Fig. 1. Tracing showing a period of gastric hunger contractions in a 9-hour old infant, before first nursing. At left end of tracing may be seen the end of the preceding hunger period. Time: 38 minutes. Chloroform manometer.

Fig. 2. Tracing showing a period of gastric hunger contractions of a 9-day-old infant, 3 hours after nursing. Note the incomplete tetanus which ends the period. Time: 45 minutes. Chloroform manometer.

The recording of the gastric hunger contractions of the new-born human infant offers no great difficulties. We used delicate rubber balloons of 15 cc. capacity, attached to a flexible rubber catheter of 2 mm. diameter. Most of the infants swallowed this apparatus without difficulty and went to sleep in our arms during the observation periods. The results were always most satisfactory with the infants asleep, as that eliminated all nervous inhibitory factors, and the disturbances from body movements and from irregularities in respiration. Practically nothing can be done with the balloon method if the infant is at all

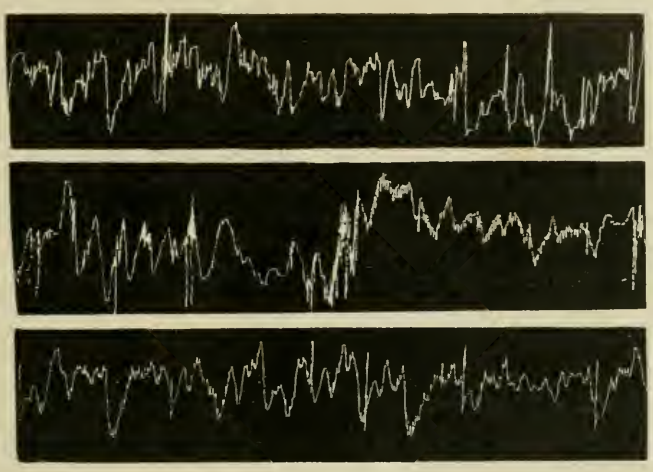

Fig. 3. Tracings of contractions of the empty stomach of a pup born 8–10 days before term. No food given before securing tracings. Time: 10 minutes. Chloroform manometer.

restless. All of our observations were made on healthy and vigorous infants.

The two premature pups were very small, and the balloon introduced into the stomach via the oesophagus had a capacity of only 4 cc.

RESULTS

1. *Human infants.* Periods of gastric tonus and hunger contractions are in evidence shortly after birth and before any food had entered the stomach. These gastric hunger periods exhibit all the peculiarities of the gastric hunger contractions of the adult, except that

the periods of motor quiescence of the stomach between the hunger periods are on the whole much shorter (10–15 minutes). When the gastric hunger contractions become very vigorous the sleeping infant may show some restlessness, and even wake up and cry. If the infant is awake the very vigorous hunger contractions frequently induce crying and restlessness. Two tracings showing typical hunger periods in a nine-hour old infant before first nursing, and in a nine-day-old infant three hours after nursing are reproduced in figures 1 and 2. The reader's attention is called to the fact that in both of these infants the gastric hunger periods end in incomplete tetanus, an index of youth and vigorous stomach.

2. *Prematurely born pups.* The observations were made before any food was given to them. The empty stomach of these very small pups exhibited a continuous motor activity of a character shown in figure 3. These contractions are not identical with the digestion peristalsis, because the latter contractions in the dog occur at 15–18 seconds intervals. The contractions shown in figure 3 last for 30 to 60 seconds or longer, and at times seem to be periods of gastric tetanus. This is the type of motor activity one might expect to observe with the slightly inflated balloon in the cardiac end and the empty stomach in very great tonus.

We are under obligations to Dr. N. S. Heaney of the Presbyterian Hospital for facilities in part of this work. We also wish to thank Mr. I. Tumpowsky and the Misses Jacobson, Rautsche, Clapp, Windmiller, and Jones for their willing assistance.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

VII. The Influence of Certain Anesthetics

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In preceding papers of this series evidence was presented that injections of adrenalin result in a hastening of the coagulation time of blood.[1] Other evidence showed that stimulation of the splanchnic nerves produces a like effect upon coagulation time.[2] Several investigators have shown that artificial stimulation of the splanchnic nerves leads to a discharge of adrenalin into the blood.[3] Also it has been proved that certain emotional reactions such as fear and rage occurring in the normal life of an animal induce a discharge of adrenalin. This latter effect has been proved to be due to the passage of impulses along the splanchnic nerves.[4] Elliot has shown that the adrenalin content of the suprarenal glands is reduced by administration of various anesthetics;[5] and that this effect with ether and chloroform is due to stimulation of the suprarenal glands through the splanchnic nerves. A similar result has been shown by another investigator, Oliva.[6] The experiments of the latter showed that chloroform discharges the adrenal glands more completely than does ether. It was also shown in the same experiments that the adrenalin content is more quickly regained after ether anesthesia than after chloroform anesthesia. The question

[1] Cannon and Gray: This Journal, 1914, xxxiv, 232.

[2] Cannon and Mendenhall: This Journal, xxxiv, p. 243.

[3] See Dreyer: This Journal, 1898–99, ii, p. 219; Tscheboksaroff: Archiv fur die gesammte Physiologie, 1910, cxxxvii, p. 103; Asher: Zentralblatt fur Physiologie, 1910, xxiv, p. 927; Kahn: Archiv fur die gesammte Physiologie, 1911, cxl, p. 240; Meltzer and Joseph: This Journal, 1912, xxix, p. xxxiv, 34; Elliott: Journal of Physiology, 1912, xliv, p. 400; Cannon and Lyman: Loc. cit., p. 377.

[4] Cannon and Mendenhall: This Journal, xxxiv, p. 255.

[5] Elliott: Loc. cit., p. 388.

[6] Oliva: Lyon Chirurg., 1914, ii, p. 11.

of the effect of anesthetics upon coagulation time has long been of prime importance to both surgeons and obstetricians. Their chief concern, however, has been in the after-effects as agents productive of post-operative or post-partum hemorrhages. Chloroform seems to be the one most often recognized as causing a change in the coagulation process. Whipple and Hurwitz[7] recently have shown that several hours after administration of large doses of chloroform to dogs the coagulation time is unchanged; they call attention, however, to the weak consistency of the clots. They ascribe the cause of post-operative hemorrhages following administration of chloroform as a failure of the clot to hold firmly rather than a retardation of clotting processes. That the liver is concerned in the coagulation of blood has been shown by many observers.

The foregoing evidence led to the question of the effects of ether and chloroform upon blood coagulation during the administration of the drugs. Inasmuch as experiments recorded in previous papers of this series were concerned with immediate factors affecting coagulation time it was thought logical to study the immediate effects of ether and chloroform upon the coagulation process. These drugs, furthermore, have been shown to exert action upon organs that are intimately involved in blood coagulation, i.e., the liver and adrenals. It was hoped, if changes occurred during anesthesia by these drugs, that such changes might be of value in studying their after-effects or in explaining after-effects of this form of anesthesia upon coagulation time, and also that they might throw some light upon the complex of organs involved in the coagulation mechanism.

The method of drawing blood and recording the coagulation time was the same as described in a previous paper.[8] Decerebrate animals (cats) were used throughout this investigation. Two reasons led to the adoption of this type of animal; first the animals of the whole series were placed under practically uniform conditions, and second, the animal was free from the anesthetic whose action it was desired to study. It was necessary in the beginning of each experiment to induce anesthesia for a short time in order to perform decerebration. Ether was used therefore in the beginning of the experiment. Care was taken to produce not too profound anesthesia and to remove the cerebrum as quickly as possible after beginning the administration of the ether. The usual routine was as follows. Simultaneously with secur-

[7] Whipple and Hurwitz: Journal of Experimental Medicine, 1911, xiii, p. 136.
[8] Cannon and Mendenhall: This Journal, xxxiv, p. 225.

ing the animal on the board the ether was applied with a cone, and the neck was prepared by clipping the hairs. By this time anesthesia was deep enough to permit operative procedures. The animal was then tracheotomized, a tracheal cannula inserted, and both carotids tied. Then it was turned over and decerebration performed according to the method described recently by Forbes and Sherrington.[9] The total time elapsing from the application of ether to its removal never exceeded fifteen minutes, usually it was from ten to twelve minutes. After decerebration the femoral artery was prepared according to directions given in a preceding paper of this series.[10] The temperature of the animal was maintained when necessary by an electric heating pad. A thermometer was inserted into the rectum. The ether or chloroform was given by means of the bottle used in ordinary laboratory operations. It consisted of a small bottle of about 75 cc. capacity. It was stoppered by a rubber cork through which passed two right angle glass tubes, each 1 cm. in diameter. One of these tubes conducted air to the surface of the anesthetic; the other conducted the ether-air mixture to the animal by means of a short rubber tube connected to the tracheal cannula. This rubber tube had an oblique cut in the wall so that by shifting the bottle more air could be mixed with the ether if the animal showed signs of asphyxia. The corneal reflex was used to determine if anesthesia was present; also vibrissae, ear and tail reflexes were used. After all operative procedures were finished the animal was left undisturbed for forty-five minutes or an hour. This was done in order that the animal might be free from ether when observations were to be made, and also because of the discovery recorded in a previous paper that operative procedures may shorten the coagulation time. It was felt that the time mentioned above sufficed to free the animal from the preliminary small dose of ether and also any hastening factor that may have been aroused by operations. All experiments began with observations taken at intervals of ten minutes for forty minutes or an hour to determine the normal coagulation time of the animal, then anesthesia was induced by the means described above and observations continued every ten minutes for an hour.

A total of sixty-three successful experiments were performed. Preliminary to the investigation of chloroform a number of experiments were made with chloral hydrate. It was thought that this drug would give some valuable data which would be indicative of the action of the

[9] Forbes and Sherrington: This Journal, 1914, xxxv, p. 367.

[10] Cannon and Mendenhall: Loc. cit., p. 227.

whole series of chlorine containing anesthetics. Moreover it might reduce the number of animals which would be necessary for the study of chloroform. Thus the fatalities resulting from the powerful toxicity of the chloroform would be reduced. In actual practice, however, the fatalities due to chloroform were surprisingly small. Inasmuch as chloral hydrate is frequently used for its anesthetic effect, the study of its influence upon coagulation is not without value.

TABLE 1

Effect of chloral hydrate

EXPERIMENT NO.	DAYS IN STOCK	SEX	DOSE IN MGMS. PER KILO	NORMAL COAGULATION TIME	PER CENT INCREASE COAG. TIME	PER CENT DECREASE COAG. TIME
1	120	Female	90	2.4	50.0	
5	¼	Male	65	2.8	60.0	
4	4	Male	65	2.8	142.0	
2	4	Female	65	3.6	30.5	
3	2	Female	65	4.0	5.0	
38	3	Male	70	6.4	10.9	
39	4	Male	70	6.5	15.3	
8	4	Female	100	6.8	0.0	0.0
42	½	Female	70	6.8	20.5	
7	3	Male	100	6.9		8.7
6	3	Male	100	7.0		7.0
41	1	Female	70	7.7		20.7
40	3	Male	80	7.9		6.3
31	2	Female	100	8.0		13.7
44	3	Female	70	8.6		6.9
43	0	Male	70	9.3		7.5
45	0	Female	80	9.3		11.8
Average				6.2	37.1	10.3

Effect of chloral hydrate. A total of twenty-three experiments were performed with chloral. Table 1 shows the results of seventeen of these experiments arranged in ascending order according to length of the normal coagulation time. The increase or decrease of coagulation time is represented in per cent of the normal. The doses were given intravenously. Injection was made slowly. It usually took three to five minutes to introduce the drug. The dose varied from 65 to 100 mgm. per kilogram. Six experiments were performed with large toxic doses (150–165 mgm. per kilogram). These are not included in the table because they are of no interest except from a toxi-

cological standpoint. Three of them showed an increase, one no change, and two a decrease in coagulation time. A glance at the table reveals the curious fact that the effect which chloral hydrate has upon coagulation bears a distinct relation to the coagulation time before chloral was administered. It is noted that if the normal coagulation time of the blood was 6.8 minutes or less, chloral prolonged the coagulation; whereas, if the normal coagulation time was 6.9 minutes or more, then chloral decreased the coagulation time. That this effect is not due to size of dosage is revealed by the table; furthermore it is unlikely that it may depend upon the sex of the animal or the length of time it had been in stock. Thus Experiment 42 was a female in stock one-half day and received a dose of 70 mgm. per kilogram. Its normal coagulation time was 6.8 minutes. Chloral increased the coagulation

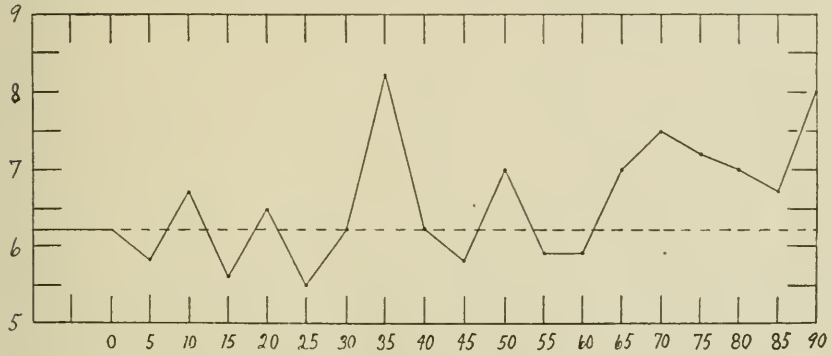

Fig. 1. Effect of chloral hydrate.

time 20.5 per cent. Experiment 41 was a female in stock one day; it received the same dose per kilogram; its normal coagulation time was 7.7 minutes. Chloral in this instance decreased the coagulation time 20.7 per cent. This shows clearly that sex and size of dosage are not the determining factors in the effect of chloral hydrate upon coagulation time. A point of further interest in the above two experiments was in the weights of the animals. It was a mere coincidence that their weights were exactly the same 2.6 kgm., and therefore each received the same size of dose of chloral. Figure 1 is a composite curve based upon the results obtained in Table 1. The straight line at the beginning of the curve represents the average normal coagulation time. It is extended as a line of dashes through the length of the curve. The ordinates represent minutes of time for coagulation to occur. The

abscissae represent intervals of five minutes from the time when the drug was given. The general averages of these experiments showed a normal coagulation time of 6.2 minutes. After chloral was administered the average coagulation time increased 4.8 per cent. Figure 2 is a composite curve constructed in the same manner as the one above. In this curve only those experiments were used whose normal coagulation time was 6.8 minutes or less. It is noted that only once did the coagulation fall below the normal, and then only 0.1 minute. The curve shows the striking effect that chloral hydrate has upon a short coagulation time. The average increase in coagulation time as shown by this curve amounted to 28.2 per cent. Figure 3 is a curve which is composed of those experiments whose normal coagulation was de-

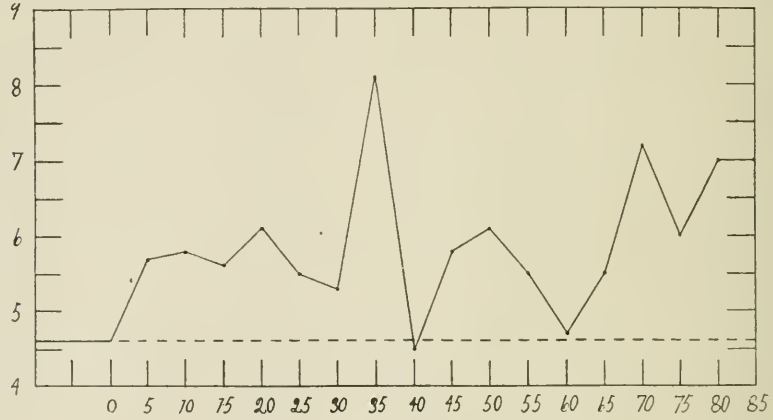

Fig. 2. Effect of chloral hydrate (short normal).

creased in these observations. The average decrease amounts to 7.5 per cent. The decrease does not correspond to the increase. In the first paper of this series the per cent of average error due to the method is stated as 6 per cent. Therefore, the result when the normal coagulation time was long might be regarded as nil. The fact that chloral hydrate exerts its retarding effect more when a short normal is present suggests the idea that it acts antagonistically to hastening factors present in the blood or that it may depress activity of organs which produce or activate hastening factors. The action of chloral hydrate upon the liver is too well known to need description here. There seems to be no reference available in regard to its effect upon the adrenals. If chloral hydrate caused the production of factors that retarded coagula-

tion one should expect it to exert its retarding effect even though the normal coagulation was long. Only one clear instance of this is shown and then a large toxic dose of chloral hydrate was used. Thus in Experiment 36 the average coagulation time for a half hour preceding the injection of chloral hydrate was 8.8 minutes; for forty minutes following the injection the average was 9.6 minutes, an increase of 9.0 per cent. This result is not far from the average per cent of error. Experiment 32 was another (normal 8.0 minutes) in which a toxic dose of the drug was used. This showed an increase of 16 per cent over the normal. The evidence in this experiment was not clear, inasmuch as the animal became extremely irritable after decerebration and because of twitching made difficult the drawing of blood. The

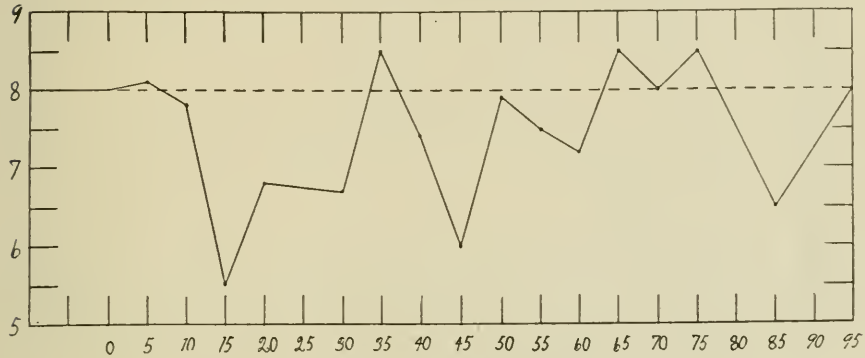

Fig. 3. Effect of chloral hydrate (long normal).

general disturbance present in this animal made the normal coagulation time doubtful. The animal became quiet after the chloral was injected. Experiment 33 was another instance in which a long normal (10.2 minutes) was increased by a toxic dose of chloral hydrate. Here again the evidence is not clear, because the animal had just come into the laboratory and in observations lasting an hour before chloral was given the coagulation time fluctuated from 6.5 minutes to 14.5 minutes. After chloral was given the respiratory center was paralyzed and artificial respiration became necessary. The increase amounted to 13.7 per cent. Contrary to this experiment is one (Exp. 37), in which a toxic dose was given to an animal whose coagulation time was short (6.0 min.). Here there was practically no change whatever. This was a male animal, in stock two days. It fought furiously while being placed upon the board and continued thus until anesthesia be-

came effective. Experiment 8 is an example of a short coagulation remaining unchanged, but the dose was not toxic. This animal, a female, had been in stock four days. The weight of evidence obtained in these experiments shows that chloral hydrate, if it affects the coagulation at all tends to prolong it. The prolongation is greatest when the normal coagulation is short. The evidence does not warrant a conclusion that retarding factors are produced.

Effect of chloroform. Fifteen experiments were performed with chloroform. Table 2 shows the results obtained. The amount of chloroform used varied somewhat, the average being 10 cc. Anesthesia, as noted by reflexes, was usually complete in from three to four minutes. With two exceptions, Experiments 58 and 50, chloroform behaved

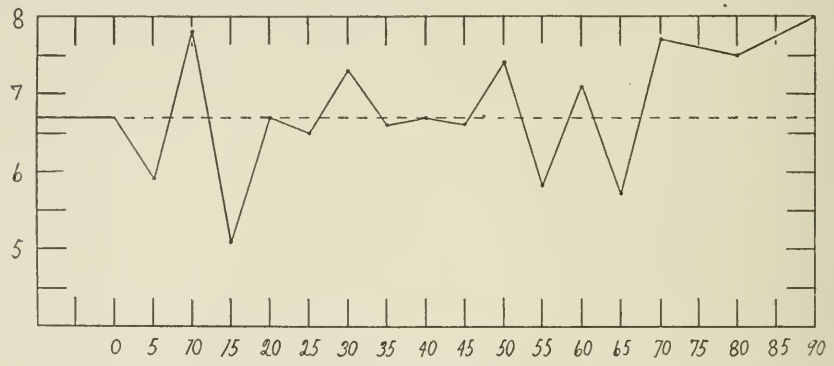

Fig. 4. Effect of chloroform.

similarly to chloral hydrate—thus if the normal coagulation time was short chloroform prolonged it, whereas, if it was long a decrease resulted. In these experiments as in those with chloral hydrate a composite curve shows little effect of the drug upon coagulation time other than to make it irregular. Figure 4 is such a curve constructed upon the basis of the observations in Table 2. The average normal coagulation time in this set of experiments with chloroform was 6.7 minutes. The average coagulation time during administration of chloroform was the same, 6.7 minutes; hence the change in per cent was 0.0. This, however, was a mere coincidence, since leaving out any one experiment would alter the figures slightly one way or the other. A curious fact is noted in the point where chloroform action changes from an increase to a decrease of coagulation time. It occurs at 7.5 minutes. With chloral hydrate it was 6.8 minutes, a difference of less than a minute.

TABLE 2

Effect of chloroform

EXPERIMENT NO.	DAYS IN STOCK	SEX	NORMAL COAGULATION TIME	PER CENT INCREASE COAG. TIME	PER CENT DECREASE COAG. TIME
52	2	Male	4.7	10.6	
63	1	Male	5.3	9.4	
58	3	Female	5.5		3.6
50	1	Female	5.8		1.7
56	2	Male	6.0	25.0	
54	1	Female	6.2	8.0	
51	2	Male	6.4	3.1	
53	3	Male	6.6	16.6	
57	1	Male	6.6	18.1	
59	3	Male	7.1	2.8	
55	2	Female	7.5	2.6	
62	1	Female	8.0		18.7
61	3	Female	8.5		8.2
60	4	Male	9.0		5.5
46	½	Male	9.2		8.6
Average			6.7	10.6	7.7

Figure 5 is a curve constructed from observations made in those experiments in which the normal coagulation time was 7.5 minutes or less.

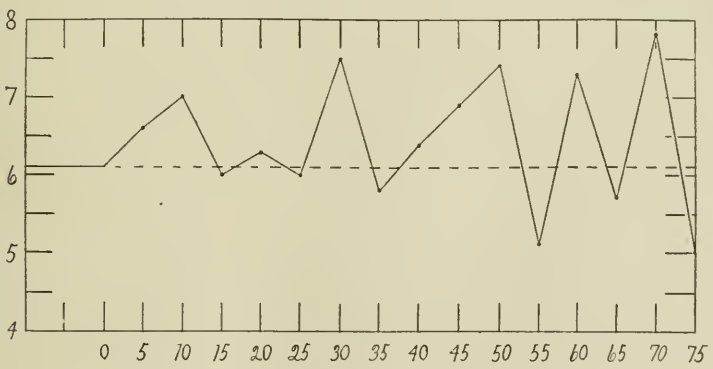

Fig. 5. Effect of chloroform (short normal).

The average normal coagulation time in these experiments was 6.1 minutes. After the chloroform was given there was an increase of 4.9 per cent. Figure 6 is a curve in which the normal coagulation time was

over 7.5 minutes. The average normal was 8.6 minutes. After the chloroform was given it decreased 11.6 per cent. The increase in coagulation time when the normal was short was not as pronounced as in the chloral hydrate experiments. In figure 5, however, there were included two experiments in which there was a decrease in coagulation time. If the average decrease is calculated in all experiments that show a decrease it is found to be 7.7 per cent; whereas, if the average increase is estimated, it is found to be 10.6 per cent. Moreover the average short coagulation normal with chloral hydrate was 4.6 minutes, while with chloroform it was 6.1 minutes. Chloroform is known to affect two organs that are important in coagulation processes, i.e.,

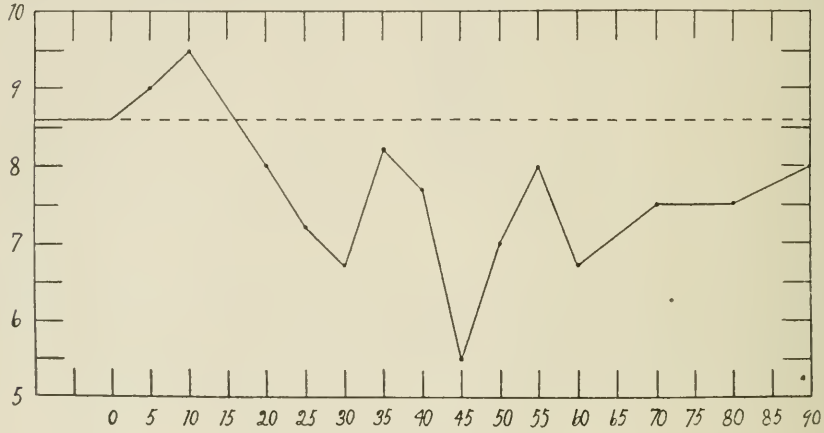

Fig. 6. Effect of chloroform (long normal).

the liver and the adrenals. It would be natural to suppose that its effect at any one time upon coagulation would depend upon various interrelations among many factors. If the adrenals were discharged completely it might still exert an effect upon coagulation by disturbance of liver function; or if the adrenals were highly charged, the resulting outpour might or might not be effective because of an impairment of liver function. The following protocol is typical of cases of chloroform anesthesia in which coagulation was prolonged.

Protocol Experiment 54.

February 10, 1915. Female cat, in stock 1 day, weight 2.5 kgm.
1.05 Placed on board and anesthetic (ether) begun.
1.15 Tracheal cannula placed and carotids tied.

1.20 Decerebration completed, ether removed.
1.40 All operative procedures completed.
2.45 Coagulation time 6.0 minutes.
2.55 Coagulation time 6.5 minutes.
3.05 Coagulation time 5.5 minutes.
3.15 Coagulation time 6.5 minutes.
3.25 Coagulation time 6.5 minutes.

Average 6.2

3.35 Chloroform administered
3.45 Coagulation time 6.0 minutes.
3.55 Coagulation time 7.0 minutes.
4.05 Coagulation time 6.0 minutes.
4.15 Coagulation time 5.0 minutes.
4.25 Coagulation time 8.0 minutes.
4.35 Coagulation time 8.5 minutes.

Average 6.7

4.36 Chloroform removed
4.45 Coagulation time 8.5 minutes.
4.55 Coagulation time 6.0 minutes.
Temperature variation in animal 1° C.
Water bath constant 25° C.

Figure 7 gives a summary of this experiment. In this experiment the increase amounts to 8.0 per cent, but the curve is typical of the increase shown by chloroform, i.e., the increase is more noticeable about an hour after the beginning of the anesthetic. Previous to the increase there may be evidence of a disturbance of balance between opposing forces with a final predominance of the factors that retard coagulation or perhaps a decrease in effectiveness of hastening factors. After the chloroform is removed the hastening factor again appears. The following protocol is of interest because of the opposite effect that is shown.

Protocol Experiment 61.

March 1, 1915. Female cat, in stock 3 days, weight 3.2 kgm.
1.10 Animal placed on board and etherization begun.
1.20 Tracheal cannula placed and carotids tied.
1.25 Decerebration completed, ether removed.
2.10 All operative procedures completed.
3.15 Coagulation time 8.5 minutes.
3.25 Coagulation time 8.5 minutes.
3.35 Coagulation time 8.5 minutes.

Average 8.5

3.36	Chloroform administered	
3.45	Coagulation time	10.0 minutes.
4.00	Coagulation time	8.0 minutes.
4.10	Coagulation time	7.5 minutes.
4.20	Coagulation time	5.5 minutes.
		Average 7.8
4.21	Chloroform removed	
5.40	Coagulation time	10.0 minutes.

Temperature variation in animal 2.5° C.
Temperature water bath constant 25° C.

The experiment is summarized in figure 8. In this experiment the average normal was 8.5 minutes. Forty-five minutes following the introduction of chloroform the coagulation time was shortened 35.2 per cent. One hour and forty minutes after chloroform was removed the coagulation time had increased over 50 per cent. The average coagulation time throughout the whole forty-five minutes of chloroform anesthesia showed a decrease of only 8.2 per cent. The evidence obtained in these experiments with chloroform shows a marked resemblance to that obtained with chloral hydrate. If the coagulation time is affected at all it is usually retarded, except when the normal coagulation time is long, when it may be decreased. The evidence does not indicate that retarding factors are produced.

Effect of ether. A total of 21 experiments were performed with ether. Thirteen were made with adrenals intact and eight with adrenals removed. Table 3 shows the results obtained in the experiments in which the adrenals were intact. In no instance did ether increase the coagulation time. An average of 50 cc. of ether was used in each experiment. The percentage decrease in coagulation time varied from 0–24 per cent. Figure 9 is a composite curve based upon the observations obtained in Table 3. The normal coagulation time was 7.2 minutes. Ether decreased it 15.2 per cent. There was no distinct relation between normal coagulation time and the effect of ether. The longest normal was 9.4 minutes; this was decreased 11.7 per cent; the shortest normal was 5.6 minutes; it was decreased 24.4 per cent. Experiments 16, 17, and 22 showed practically no change, but these may readily be explained. In Experiment 16 the animal had been in stock only 12 hours. During the first 35 minutes of ether anesthesia the coagulation time increased, reaching 37.0 per cent above the normal; twenty-five minutes later the coagulation time was 19.3 per cent shorter than normal. It continued with some variation for over an hour.

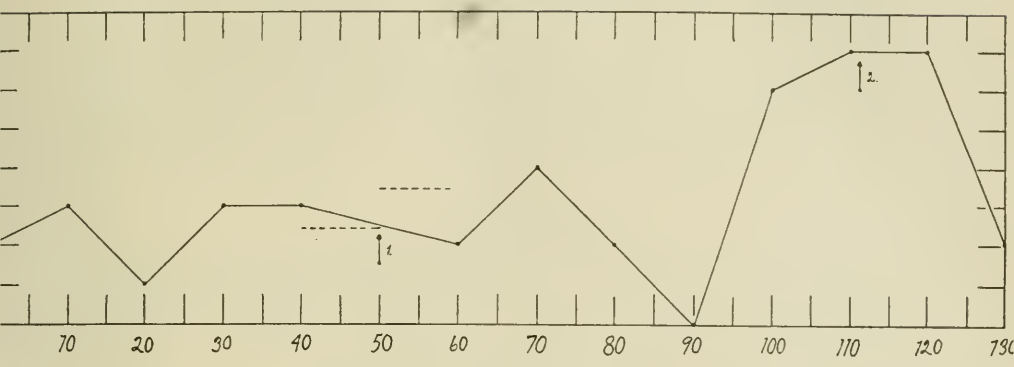

Fig. 7. A summary of Experiment 54. Chloroform was given at 1, removed at 2. Dotted lines resent averages of coagulation time before and after chloroform was given.

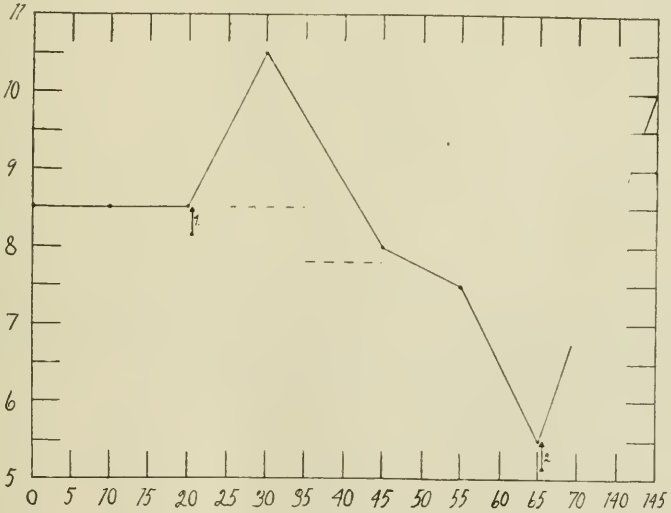

Fig. 8. Summary of Experiment 61. Chloroform given at 1, removed at 2. Dotted lines represent averages before and after chloroform was given.

This caused the final average to appear the same as the normal, when the real effect was a marked diminution. Experiment 17 showed a similar initial rise followed by a drop in the coagulation time. The animal had been in stock 20 hours. In Experiment 22 the animal died suddenly one hour after the ether was started. The coagulation time was irregular throughout the experiment. The following protocol is typical of the ether effect.

TABLE 3

Effect of ether (adrenals intact)

EXPERIMENT NO.	DAYS IN STOCK	SEX	NORMAL COAGULATION TIME	PER CENT INCREASE COAO. TIME	PER CENT DECREASE COAG. TIME
19	3	Male	5.6		24.4
14	2	Female	6.1		9.8
16	½	Male	6.2		
17	¾	Male	6.3		3.1
21	1	Female	6.5		7.6
12	150	Female	6.8		13.2
13	150	Female	6.8		17.6
11	150	Female	7.1		23.9
18	2	Female	7.6		21.0
15	2	Female	8.5		15.3
22	2	Female	8.8		1.1
20	6	Male	9.1		8.7
10	?	Male	9.4		11.7
Average			7.2	0.0	15.2

Protocol Experiment 15.

November 11, 1914. Animal in stock 2 days, female, weight 2.4 kgm.
1.50 Animal placed on board, etherization begun.
1.55 Tracheal cannula placed and carotids tied.
2.00 Decerebration completed, ether removed.
2.15 Operations completed.
3.00 Coagulation time 7.5 minutes.
3.10 Coagulation time 8.0 minutes.
3.20 Coagulation time 10.0 minutes.
3.35 Coagulation time 8.5 minutes.
3.45 Coagulation time 8.5 minutes.
3.55 Coagulation time 8.5 minutes.

Average 8.5

4.00 Ether
4.05 Coagulation time 9.5 minutes.
4.15 Coagulation time 6.5 minutes.
4.25 Coagulation time 7.0 minutes.
4.35 Coagulation time 8.5 minutes.
4.45 Coagulation time 6.5 minutes.
4.55 Coagulation time 6.5 minutes.
5.05 Coagulation time 7.0 minutes.
5.15 Coagulation time 6.5 minutes.

Average 7.2

Animal's temperature showed variation of 1.0° C.
Temperature of water bath constant 25° C.

TABLE 4

Effect of ether (adrenals removed)

EXPERIMENT NO.	DAYS IN STOCK	SEX	NORMAL COAGULATION TIME	PER CENT INCREASE COAGULATION TIME	PER CENT DECREASE COAGULATION TIME
25	4	Male	5.9	13.5	
24	3	Female	6.9		2.8
29	0	Male	7.2		3.7
28	2	Male	7.3		8.2
27	5	Male	7.3		
30	5	Female	7.9		1.2
23	3	Male	8.7		6.8
26	0	Male	9.3		7.5
Average			7.5		1.3

Figure 10 shows a summary of the experiment.

The consistent action of ether suggested the idea that only one factor of coagulation, the hastening factor, was affected. Since Elliott has shown that ether discharges the adrenal gland it was thought desirable to remove the adrenals and see if adrenalin was the factor which was affected. Table 4 shows the results of 8 experiments with adrenals removed. The results are interesting in that one shows an increase of 13.5 per cent. This was the only experiment with ether that showed an increased coagulation time. The remaining experiments all showed a decrease, but the decrease was so slight that it was practically nil. All might easily fall within the range of per cent of error. Figure 11 is a composite curve based upon observations recorded in Table 4. The effect of removal of adrenals is clearly shown by comparing this

Fig. 9. Effect of ether (adrenals intact).

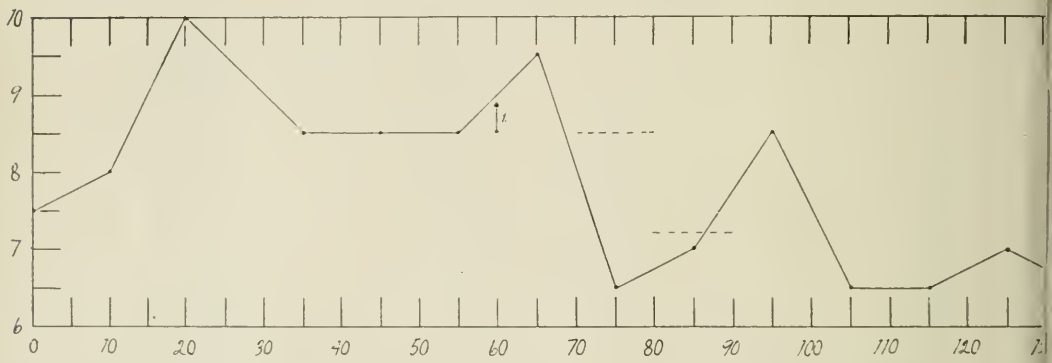

Fig. 10. Summary of Experiment 15. Ether given at 1. Dotted lines represent averages befor after ether was given.

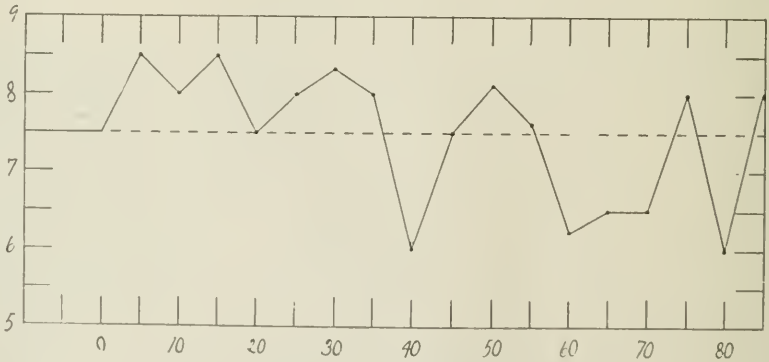

Fig. 11. Effect of ether (adrenals removed).

curve with figure 9 where the adrenals are intact. The average decrease per cent with adrenals out was 1.3 per cent. The evidence shows therefore that ether decreases the coagulation time and that the effect is exerted through the adrenal glands.

Since the experiments with chloral hydrate and chloroform show so much irregularity it is conceivable that more than one organ is involved by them in the coagulation changes. That both drugs affect the liver is well known and that chloroform discharges the adrenal glands has been described by two investigators. The action of chloral hydrate upon coagulation time points to involvement of a hastening and a retarding process. Possibly the adrenals are discharged by chloral hydrate, just as they are by chloroform. It was not shown in these experiments that chloral hydrate or chloroform produces or stimulates the production of a factor retarding coagulation. The experiments in which increase in coagulation time was shown might well be explained in another way. For example, in Experiment 37 mentioned above, the normal coagulation was 6.0 minutes. A toxic dose of chloral hydrate produced practically no change in the coagulation time. This seems to add additional evidence to support the suggestion made in a previous paper that a hastening process may involve the action of a factor upon the liver (or intestine).[11] It may readily be conceived here that chloral hydrate discharged the adrenal gland, and at the same time rendered the liver incapable of being acted upon by the adrenal secretion. As a result no hastening of coagulation occurred. The animal in this experiment was a male in stock two days and had fought vigorously. He might easily have largely discharged his adrenal glands so that no more was discharged by chloral hydrate. This would have left chloral hydrate free to stimulate at least temporarily the production of a retarding factor if it had such power. The evidence, however, is negative. Thus the coagulation time before the introduction of chloral hydrate was as follows: 6.0, 5.5, 5.5; afterward it was 6.0, 5.5, 6.5, 5.5, 6.0. The actions of chloroform, which is known to discharge adrenalin (a hastening factor), may well be explained in terms of the factor of adrenalin and its action upon a susceptible or non-susceptible liver. Chloroform showed usually a retarding influence upon coagulation. This may have been due to its action upon the liver. Thus it may stop the action of adrenalin upon the liver and in that way remove the factor that was keeping the coagulation time

[11] Cannon and Mendenhall: Loc. cit., p. 250.

low. In figure 7 above it is seen that the prolongation did not occur until several minutes after the chloroform was given. Ten minutes after the chloroform was removed the coagulation process was still prolonged, but in the following ten minutes it returned to normal. This may be explained by the liver again becoming susceptible to the action of adrenalin. The decrease in coagulation shown by chloroform may be explained in terms of the adrenalin factor also. Thus in figure 8 above, the animal had been in stock three days, had become quiet, and naturally the hastening factor, adrenalin, was not being discharged. Its coagulation time was consequently long, 8.5 minutes. It has been shown that a *large* amount of adrenalin may produce first a retardation and then an acceleration of coagulation.[12] The first

TABLE 5

Summary of data

TOTAL NO. EXPERIMENTS	NORMAL COAGULATION TIME	DRUG	PER CENT INCREASE COAGULATION TIME	PER CENT DECREASE COAGULATION TIME
17	6.2	Chloral hydrate	4.8	
9	4.6	Chloral hydrate	28.2	
8	8.0	Chloral hydrate		7.5
15	6.7	Chloroform		
11	6.1	Chloroform	4.9	
4	8.6	Chloroform		11.6
13	7.2	Ether (adrenals intact)		15.2
8	7.5	Ether (adrenals removed)		1.3

application of chloroform may have discharged an amount of adrenalin which acted upon the liver and produced the *retarding* factors before the liver became insusceptible to the action of adrenalin. As the chloroform was administered it continued to discharge adrenalin and when it was removed and the liver again became susceptible to its action it acted the same as a large dose of adrenalin at any time. It prolonged the coagulation time.

The evidence with ether points clearly toward involvement of one factor of coagulation, adrenalin. It might be regarded as a bloodless method of injecting adrenalin intravenously. Thus in Experiment 16 above the preliminary rise is typical of a large dose of adrenalin and

[12] Cannon and Gray: Loc. cit., p. 238.

the subsequent fall is typical. Experiment 17 is a similar effect. This preliminary rise frequently caused the final average to appear smaller than it actually was. That adrenalin is the factor involved is shown in the experiments in which the adrenals were removed. That most of them still showed a decrease may easily be due to discharge from accessory chromaffin tissues. Some control experiments in which nothing was given showed that the coagulation time decreased slightly as the experiment proceeded. No explanation is offered for the one instance in which ether prolonged the coagulation time when the adrenals were excluded. Table 5 shows briefly the results obtained in all the experiments.

SUMMARY.

The observations in these experiments seem to warrant the following conclusions.

1. Coagulation time is little altered by chloral hydrate unless it is normally short, then it is prolonged.

2. Coagulation time is affected by chloroform as by chloral hydrate, i.e., if the process is affected at all it more usually is prolonged rather than hastened.

3. The effects of chloral hydrate and chloroform are probably the result of disturbance and consequent interaction between two or more organs which are important in the coagulation process; probably liver (intestine?) and adrenal glands.

4. The evidence is not sufficient to prove that a retarding agent is produced.

5. Coagulation processes are hastened by ether anesthesia.

6. The effect of ether is exerted wholly through its action upon the adrenals.

A CALORIMETRIC CALIBRATION OF THE KROGH BICYCLE ERGOMETER

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Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.

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A quantitative study of the relation between muscular work and total metabolism requires the use of some form of apparatus which will transform the muscular work into measurable units. In a considerable part of the earlier research on this problem with man, the arms have been employed to rotate ergometers of various forms. Much valuable research has been carried out by Zuntz and his associates with a piece of gymnasium apparatus designated by them as the Gaertner ergostat, with the brake ergometer of Zuntz,[1] and with the dynamometer of Fick.[2] Johansson, Tigerstedt, and their associates have also made studies with Johansson's extraordinarily ingenious ergostat.[3]

In recent years the use of some form of bicycle has been recognized as affording the most practical method for securing large amounts of external muscular work and at the same time obtaining a relative fixation of the body of the subject, so as to permit the measurement of the gaseous metabolism by some type of breathing appliance. One of the earliest uses of the bicycle form of ergometer was that made by Atwater and Benedict[4] in which a small pulley attached to the armature shaft of a generator pressed against the rear wheel of a stationary bicycle. When the subject was pedaling, the friction between the wheel and the pulley developed an electric current in the armature which could be measured. Subsequently a special type of bicycle ergometer was devised on the electric brake principle, which was used in a series of investigations with the respiration calorimeter at Wesleyan University,

[1] Zuntz: Archiv für Physiologie, 1899, p. 372.

[2] Fick: Archiv für die gesammte Physiologie, 1891, l, p. 189.

[3] Johansson: Skandinavisches Archiv für Physiologie, 1901, xi, p. 273.

[4] Atwater and Benedict: U. S. Department of Agriculture, Office of Experiment Stations, Bulletin 109, 1902, p. 20.

Middletown, Connecticut, in studying the effective work produced by several bicycle riders. The apparatus was briefly described in a publication by Benedict and Carpenter;[5] more recently a detailed description of the apparatus, together with a series of interesting calorimetric calibrations made with it, was published by Benedict and Cady.[6]

Bowen[7] published in 1903 a description of an ergometer in which the rear wheel of a bicycle was replaced by a grindstone, weighing about 50 kilograms, the apparatus being also supplied with a brake. Later some researches made with this ergometer were published.[8]

As a means for securing a considerable amount of muscular work, Haldane fitted up a tricycle, weighting the wheels heavily to convert them into fly wheels, and using a brake consisting of a strap passed around the brake-wheel. The records were made on a spring balance.[9]

Zuntz exhibited an apparatus at the Dresden Hygiene Congress in 1911 in which a brake was applied to a bicycle ergometer fashioned much after the design of the Zuntz brake ergometer. To our knowledge no description of this apparatus has thus far been published. Personal inspection of it by one of us showed that it is very well constructed and should give most satisfactory results.

Amar also used a bicycle, the rear wheel being supplied with a special weight attached with a broad steel band. He adapted a brake to the instrument and connected it with either a dial dynamometer or a balance.[10]

Boussaguet likewise used a bicycle, fitted with a Prony brake, in studying the work of miners.[11]

More recently Martin[12] has described a simple and convenient form of bicycle ergometer, maintaining that the instrument has an error of less than 1.0 per cent. A special cast iron wheel is used in place

[5] Benedict and Carpenter: U. S. Department of Agriculture, Office of Experiment Stations, Bulletin 208, 1909, p. 11.

[6] Benedict and Cady: Carnegie Institution of Washington Publication No. 167, 1912; Cady and Benedict: Physikalische Zeitschrift, 1912, xiii, p. 920.

[7] Bowen: Contributions to Medical Research dedicated to Victor Clarence Vaughan, June, 1903, p. 462.

[8] Higley and Bowen: This Journal, 1904, xii, p. 311.

[9] Haldane: Journal of Physiology, 1905, xxxii, p. 225.

[10] Amar: Le rendement de la machine humaine, Paris, 1910, p. 30; Journal de Physiologie et de Pathologie générale, xiv, p. 298, 1912; Le moteur humain, Paris, 1914, p. 390.

[11] Boussaguet: Recherches expérimentales sur les conditions physiologiques du travail des mineurs. Thèse, Paris, 1912.

[12] Martin: Journal of Physiology, 1914, xlviii, p. xv.

of the rear wheel of the bicycle; a fabric band is wrapped around this wheel and connected with two spring balances which indicate the amount of tension. As yet we have seen no publication of researches carried out with this ergometer.

Finally Krogh[13] of Copenhagen has modified and greatly improved the electric brake bicycle ergometer. He has most ingeniously applied the electric brake principle and has also been able to weigh the work done with the apparatus. Krogh's instrument, which was first exhibited at the International Congress in Groningen, appeared to be so well adapted for quantitative studies of the relation between muscular work and metabolism that it was secured for the Nutrition Laboratory.

With all forms of electric brake apparatus there is always an element of uncertainty as to the variations in the mechanical friction with load. Furthermore, there is always present the possibility of the distortion of the magnetic field as a result of the rotation of the copper disk. With the original form of ergometer it was observed that there was a most singular calibration curve and that the amount of work done was not directly proportional to the number of revolutions, since the heat per revolution varied considerably with different speeds. The wholly unexpected calibration curves found by Benedict and Cady[14] have made all who attempt to work with this type of apparatus somewhat uncertain as to the exact values to be obtained at different speeds. Theoretically, the electric brake ergometer of Krogh eliminates in large part all of the errors incidental to the earlier form of the bicycle ergometer; nevertheless it seemed necessary to calibrate the new instrument carefully, preferably in heat units.

When an attempt was made to calibrate the first electric brake ergometer, it was suggested by Professor Atwater that the instrument be placed inside of the respiration calorimeter and rotated by means of a shaft and motor outside of the calorimeter chamber. The amount of heat generated per revolution could then be directly determined by the calorimeter. This method of calibration proved most satisfactory and a series of tests made with the calorimeter at Middletown and a few years later with the chair calorimeter in the Nutrition Laboratory showed a remarkably close agreement.

Since ultimately in metabolism studies a knowledge of the calories resulting from muscular work is particularly desired, the measurement

[13] Krogh: Skandinavisches Archiv für Physiologie, 1913, xxx, p. 375.

[14] Benedict and Cady: loc. cit.

in heat units of the work done has distinct advantages. Furthermore such a method of calibration takes into account all errors due to friction which cannot be estimated by ordinary methods. The friction tests with the first form of ergometer showed that in all probability, with a well constructed bicycle, using sprocket wheels and roller bearing chains, the friction was practically negligible. Nevertheless, the uncertainty as to the exact values obtained with the ergometer is well pointed out by Krogh in discussing the sources of error in the apparatus. After conference with Dr. Krogh it was decided that the instrument would probably prove of such general application for muscular work experiments that its calibration in one of the respiration calorimeters of the Nutrition Laboratory would be highly desirable. Accordingly we mounted the apparatus in a large calorimeter and have made a series of tests with it which are described in this paper.

The Krogh ergometer consists of an ordinary bicycle from which the front wheel is removed. The rear wheel is replaced by a copper disk having a lead ring around the periphery, this weighted wheel acting more or less as a fly wheel. The four magnets, instead of being mounted permanently as in the original bicycle ergometer, are mounted on a metal frame which is attached with ball bearings to a prolongation of the rear axle of the bicycle. A side view of the apparatus, as partially dismantled and mounted in the chair calorimeter, is shown in figure 1. In order to save space in the chamber, the front handle bars and fork have been removed. Another view of the apparatus, as seen from above, is shown in figure 2. For further details regarding the apparatus, reference must be made to the detailed and diagrammatic figures given by Krogh in his description.

To simplify calculations the exact distance between the centre of the axle to the point of suspension of the pan upon which the weights are placed has been made equal to 0.3182 meters. By means of a screw counterpoise the weight of the arm and pan can be accurately compensated and the whole system brought into perfect equilibrium. If, then, a weight is placed upon the pan and a current passed through the fields, when the disk is rotated the pan and weight will be elevated and suspended free in the air. With a considerable strength of field current there will be a drag upon the magnets which will ultimately raise a weight as large as 6 kilograms and hold it in equilibrium. Under these conditions the work per revolution will be represented by the weight on the pan multiplied by the circumference of a circle of which the hub of the wheel is the centre and the point at which the pan is

suspended is on the circumference. The ergometer is so constructed that the circumference of this circle is exactly 2 meters. Thus, for every revolution of the wheel with a weight of 1 kilogram on the pan, there will be 2 kilogrammeters of work performed.

Krogh first depended upon hand regulation for adjusting the cur-

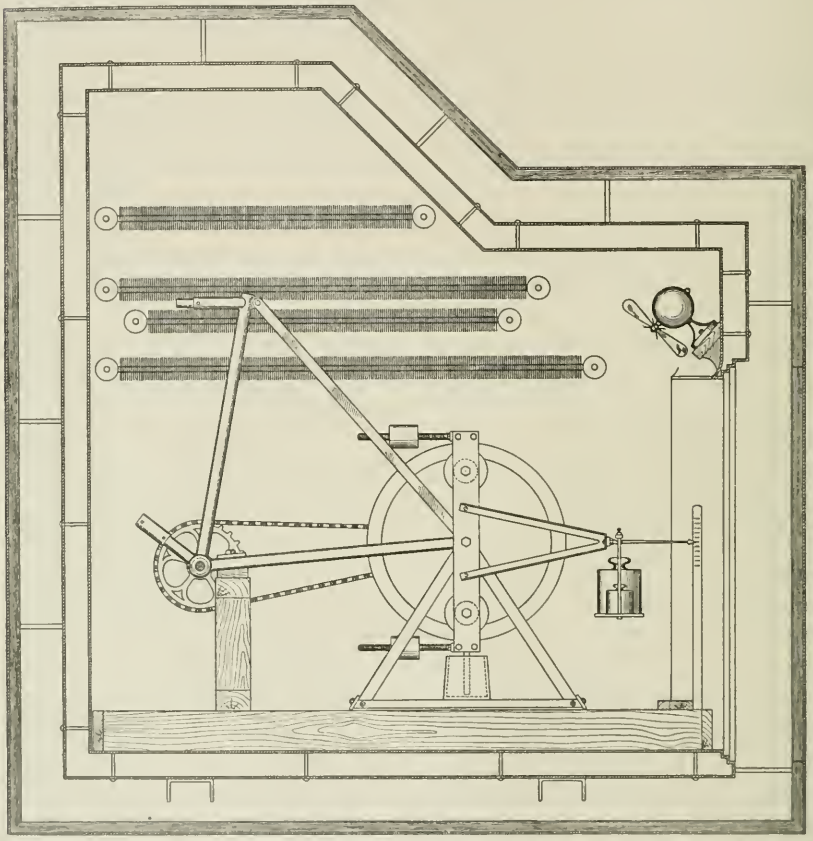

Fig. 1. Krogh bicycle ergometer as mounted for calibration in the chair calorimeter. (Side view.)

rents through the magnets to hold the balance system in equilibrium and while he subsequently applied an automatic arrangement, hand regulation was used in all of our tests. To prevent gross movements of the system due to the irregularity of pedaling by a man, Krogh has also added a damping device which consists of a heavy sheet of

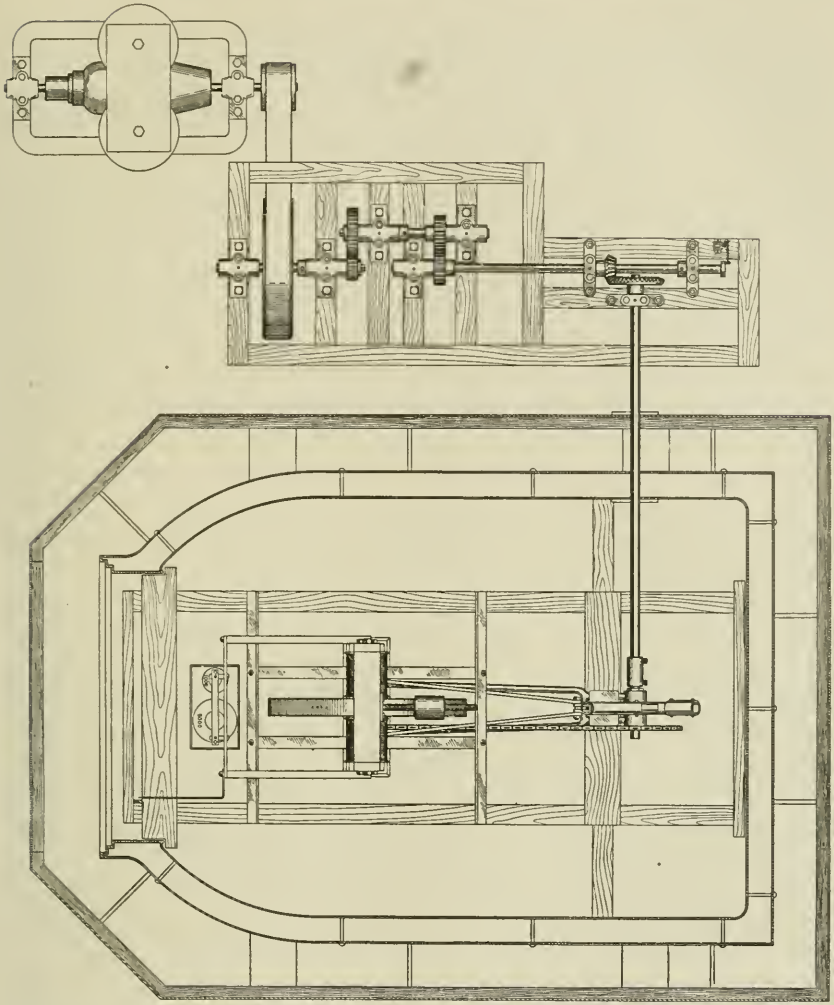

Fig. 2. Krogh bicycle ergometer as mounted for calibration in the chair calorimeter. (View from above.)

lead attached to the magnet frame. This lead paddle dips in a trough containing a thick syrup solution. While this damping device is of advantage in the practical use of the apparatus, in calibrations of the ergometer in which the apparatus is driven by an electric motor, irregularities of movement do not exist and hence it was unnecessary to employ the damping device in the calibrations.

The Krogh apparatus permits very considerable variations in the amount of work performed. Thus Krogh has shown that as large an amount of work as 2900 kilogrammeters per minute may readily be done upon the apparatus. It seemed desirable in our observations, therefore, to calibrate over a considerable range and we were at once confronted by the fact that while the chair calorimeter, which was used for these calibrations, is normally not used for experiments in which the total heat production—that of a sitting man—is much, if any, over 125 calories per hour, here we had to consider the possible production of 6 large calories per minute, exclusive of the heat developed in the fields of the magnets by the magnetizing current and the heat due to a small electric fan, which is used in securing temperature equilibrium inside the chamber. A more extended system of heat absorbers was therefore required for these tests and accordingly the ordinary heat absorbing system, which is shown in the original description of the chair calorimeter,[15] was supplemented by several lengths of heat absorbing coil, making a total length of 8.7 meters. The disposition of these extra coils is clearly shown in figure 1.

Under these changed conditions, it was necessary to test the respiration chamber calorimetrically by developing 6 calories per minute electrically inside the chamber. We therefore checked all of the calibrations of the ergometer by making electrical check tests in which approximately the same amount of heat was developed as was actually developed in the ergometer calibration. The electrical check tests were usually three to five hours in length and the heat measured varied from 150 to 350 calories per hour. The results showed that as a calorimeter the apparatus had a very high degree of accuracy.

In mounting the apparatus it was of course necessary to have the driving mechanism outside of the chamber and for this purpose a shaft was carried through the copper wall[16] of the calorimeter and attached directly to the pedal bearing of the ergometer. By means of a system of reduction gears, which varied somewhat with the speeds employed, this shaft was connected with a 2 kilowatts electric motor outside of the calorimeter. The connection between the motor, gearing, and the ergometer is shown in figure 2. A revolution counter attached to

[15] Benedict and Carpenter: Carnegie Institution of Washington Publication No. 123, 1909.

[16] To prevent interchange of heat along the shaft the temperature of the calorimeter laboratory was always kept the same as that of the interior of the calorimeter.

the reduction gear shaft recorded the number of revolutions of the pedal per minute. The weights on the swinging pan of the ergometer could be varied as desired. The arm of the pan was projected somewhat by the pointer which was held in equilibrium by variable resistance outside the chamber in parallel with the magnets.

To equalize temperature distribution inside the chamber, an electric fan has been found advantageous in practically all of our work with the calorimeters, and was retained in the calibration tests. The heat brought away by the cooling water current of the calorimeter included the heat developed as a result of the mechanical work, plus the electric energy required to magnetize the fields and the heat developed by the fan. In our final computations these two latter factors are deducted. Throughout the entire series of calibration tests, frequent records were

TABLE 1

Results of calibration test of Krogh bicycle ergometer, December 10, 1914

TIME	WEIGHT OF WATER	CORRECTED TEMPERATURE DIFFERENCE	TOTAL HEAT MEASURED	CORRECTION FOR CHANGE IN TEMPERATURE OF CALORIMETER	HEAT DUE TO CURRENTS IN MAGNETS AND FAN	CORRECTED HEAT MEASURED	TOTAL NUMBER OF REVOLUTIONS OF DISK	HEAT PER REVOLUTION
p. m.	*kilos.*	*°C.*	*cals.*	*cals.*	*cals.*	*cals.*		*cals.*
12.26–1.26	80.67	4.432	357.5	−1.0	85.8	270.7	9627	0.02812
1.26–2.26	80.65	4.310	347.6	−0.4	90.7	256.5	9024	0.02842
2.26–3.26	80.62	4.242	342.0	+0.8	92.7	250.1	8874	0.02818

made of the voltage and current on the fan and likewise that required to magnetize the fields of the ergometer. With a rate of revolution so constant as that obtained with an electric motor, the variations in the magnetizing current would be very slight and readings every four minutes could be relied upon to give an average value throughout the experiment.

The results of a specimen experiment are given in Table 1. In this experiment the load on the pan was 6 kilograms. There were 47.0 revolutions per minute of the pedals, which corresponded to 152.9 revolutions per minute of the disk or rear wheel, as the ratio of the pedals to the rear wheel is 1 to 3.25. This ratio was obtained by the number of teeth on the sprockets, the large sprocket having 26 teeth and the smaller sprocket 8 teeth. In the first column of the table is given the time for each period; in the second the weight of water passing through the cooling system; in the third the temperature difference corrected

for pressure on the thermometer bulbs; and in the fourth the total calories measured. Slight capacity corrections are shown in column five, the heat required for magnetizing the fields and for the fan is given in column six, and in column seven is given the corrected measurement, i.e., the total calories measured less the capacity correction and the heat due to the magnetizing of the fields and to the fan. The total number of revolutions of the disk or rear wheel is given in the next to the last column and finally the calories per revolution are computed.

It will be seen that in the three 1-hour periods the heat measured per revolution was 0.02812, 0.02842, and 0.02818 large calorie respec-

TABLE 2

Results of calibration tests of the Krogh bicycle ergometer, made with the chair calorimeter

DATE	DURATION OF TEST	LOAD	REVOLUTIONS PER MIN. OF PEDALS	REVOLUTIONS PER MIN. OF DISK	HEAT PER REVOLUTION OF DISK		
					Found	Theory	Ratio of found to theory
1914	*hrs.*	*kilos.*			*cals.*	*cals.*	*per cent*
Dec. 10	3	6	47.0	152.9	0.02824	0.02813	100.4
Dec. 14	4	6	43.4	141.2	0.02828	0.02813	100.5
Dec. 16	3	6	58.2	189.2	0.02814	0.02813	100.0
Dec. 17	3	3	124.6	405.1	0.01414	0.01406	100.6
Dec. 31	3	3	84.3	274.1	0.01406	0.01406	100.0
1915							
Jan. 1	3	2	144.5	469.7	0.00946	0.00938	100.9
Jan. 4	3	4	82.7	268.8	0.01880	0.01875	100.3
Jan. 8	4	5	70.1	227.9	0.02351	0.02344	100.3
Jan. 9	4	1	144.3	469.1	0.00479	0.00469	102.1
Jan. 11	4	1	105.3	342.2	0.00471	0.00469	100.4

tively, with an average of 0.02824 large calorie. Since each revolution of the disk was equivalent to the load (6 kilograms) multiplied by the circumference of the circle (2 meters), we have here 12 kilogrammeters as the amount of work done. Using 426 6 kilogrammeters[17] as the equivalent of 1 large calorie, we find that 12 kilogrammeters correspond to 0.02813 large calorie. Thus in this particular experiment we find that the ergometer produced 100.4 per cent of the theoretical energy computed from the weight on the pan and the revolutions of the disk.

The results of all of the ergometer calibrations have been summarized in Table 2. The tests included calibrations with weights on the pan

[17] Armsby: Principles of animal nutrition, New York, 1906, p. 233.

ranging from 1 to 6 kilograms and with revolutions of the pedals per minute varying from 43.4 to 144.5 and of the disk or rear wheel from 141.2 to 469.7. In other words, the tests include great varieties of speed and weight, extending quite outside the range of ordinary physiological experimentation. It will be seen that the percentage of theory found with this ergometer is striking, the average of the 10 tests being 100.55 per cent with variations from this value exceeding 1 per cent in only one instance. Since the rate of speed and the load were so greatly varied, it is reasonable to conclude that the friction plays a wholly insignificant rôle with this instrument and may accordingly be entirely neglected in any computations of the work done. With the Krogh ergometer, it is therefore justifiable to use without corrections the formula

$$W = 2pR$$

in which W is the work in kilogrammeters, p is the weight in kilograms and R the number of revolutions of the rear wheel.

SUMMARY.

A Krogh electric brake bicycle ergometer was placed inside of a bed calorimeter and rotated from the outside by means of an electric motor. The heat developed was measured by the calorimeter and has been compared with the computation of the work done in sustaining various loads on a suspended balance pan. These experiments showed that friction and other extraneous factors may be entirely neglected in using the Krogh bicycle ergometer and that the results obtained by calibration were within 0.5 per cent of theory. The experiments included tests at different rates of speed and with different weights on the balance pan.

THE EFFECT OF ADRENALIN ON THE HEART-RATE

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In a previous article[1] we have attempted to determine some of the mechanisms by which the immediate increase in pulse-rate at the beginning of a period of exercise is brought about. Our conclusions were that the increase in rate is produced principally by a decrease in vagal tone. Although acceleration may take place through the accelerators the evidence seemed to indicate that they act chiefly in maintaining the resting pulse-rate and that their accelerating action is a factor of safety superimposed on the more labile vagus mechanism. Incidental to this work we were able to make some observations on the action of the adrenals.

Animals with the accelerators removed and the vagi cut did not show a noticeable increase in heart-rate unless there were symptoms of asphyxia. Soon after the vagotomy when the signs of respiratory distress were most marked, exercise produced a great increase in pulse-rate. In the succeeding days as the signs of asphyxia decreased the exercise acceleration grew very much less. That the marked increase of rate had been due to the asphyxial secretion of the adrenals we proved by ligating these organs. Since the adrenalin seemed to be a factor only when exercise was pushed to an extreme we concluded that in the normal animal with the vagi intact the secretion of adrenalin could hardly be considered as one of the mechanisms which immediately increase the heart-rate on exercise. We still believe this holds for moderate exercise when uncomplicated by emotional excitement.

It is to be noted that our argument does not rest on the fact that adrenalin as usually given experimentally produces cardiac inhibition if the vagi are intact, but that the conclusions are based on our direct experiments. In our discussion we did overlook the fact however that as exercise increased in amount and the vagus tone grew less and less

[1] Gasser and Meek: This Journal, 1914, xxiv, p. 48.

there must of course come a time when any secretion of adrenalin might have an accelerating effect on the heart-rate.

Recently Hoskins and Lovellette[2] have objected to any conclusions that adrenalin does not always cause acceleration in exercise on both theoretical and experimental grounds. They point out in the first place that the doses of adrenalin experimentally shown to give inhibitory effects have been far in excess of the physiological secretions determined by their previous work. The rate of injection too has been much more rapid than the normal adrenal discharge. There is no gainsaying these important and just criticisms which, however, we feel do not apply to the experimental part of our work.

In the second place Hoskins and Lovellette present a table of 28 experiments on dogs in 18 of which there is increased heart-rate following the injection of adrenalin in physiological amounts. An examination of their data shows that the initial pulse-rate was in all cases very high. The lowest rate recorded is 144 per minute and the highest 258. The average is 188 per minute. This indicates a low vagal tone which was to be expected from the ether anaesthesia. It is by no means surprising that under these conditions 18 of the dogs showed an increase in heart-rate with small doses of adrenalin. That the heart itself is exquisitely sensitive to adrenalin we have had occasion to observe in work on the isolated heart. As soon then as the vagus mechanism becomes sufficiently depressed and the secretion of adrenalin becomes sufficiently large stimulation of the heart would of course result. The animals of Hoskins and Lovellette represent therefore to our mind a picture of extreme exercise, the decrease in vagal tone in their case being due to the ether anaesthesia. Limited to such conditions their conclusions are eminently correct. Adrenalin in a time of extreme muscular exertion when the decrease in vagal tone has done all the accelerating it can, is then able to show its direct stimulating effect on the heart muscle.

Although our previous work seemed to show that in dogs the adrenals were not concerned in any large degree in the increased pulse-rate which resulted from two minutes running, it has seemed worth while to study directly the effect of adrenalin when injected in small amounts into the unanaesthetized animal.

To accomplish this the dogs were laid on the floor and held until they had become perfectly quiet. The injection was made into an ear vein by a hypodermic needle which was connected with a burette,

[2] Hoskins and Lovellette: Journal of the American Medical Association, 1914, lxiii, p. 316.

the fluid being under air pressure in the burette. By means of this pressure and the proper opening of the burette cock, the injection could be made absolutely regular and at any desired speed. Usually the needle could be inserted without the dog even moving. In case the animal flinched at the prick of the needle, it was again quieted before the experiment proceeded. The right front and left hind leg of the dog were connected to the string galvanometer and electrocardiographic records of the heart-beat were taken at one-half intervals immediately before, during and from two to four minutes after the injection ceased. Each record was at least one respiratory cycle long, usually several being included, in order to avoid respiratory variations obscuring the count.

Adrenalin hydrochlorid and crystalline adrenalin were used. The amount injected varied from 2–3 cc. and the strength from 1: 50,000 to 1: 500,000. The duration of the injections varied from 1 minute 20 seconds to 3 minutes 55 seconds. These quantities were chosen so that the injection might simulate in some degree at least the physiological secretion.

The results of our experiments are presented in Table 1. It will be seen that in 25 injections on nine different dogs, in all but two the heart was slower at the end of the injection than at the beginning. Even these two cases are really not exceptions for in experiment 5^3 the rate was as low as 55 per minute during the second minute of the injection. By the end of the injection it had however risen to its former level. In experiment 9^3 also the rate at the end of the first minute had dropped to 102 but by the end of the injection it had risen to 132 per minute. In all cases then a slow injection of adrenalin in doses approximating its physiological secretion has given us in the intact resting animal a slowing of the pulse.

According to the generally accepted belief that this slowing takes place as a reflex through the vagus, we should expect less effect in those cases with a depressed vagus mechanism, i.e., those hearts with a high rate due to loss of vagal tone. The data bear this out in a few instances rather definitely. Variations in dose and time of injection make only a few comparisons possible, but in experiment 3 it may be noted that a rate of 150 was reduced only to 141 while under almost identical conditions a rate of 129 fell to 108.

The pulse rates for the successive half minutes show that the effect of adrenalin in these doses comes on in from one-half to one minute after the injection starts. With the higher dilutions particularly the

pulse-rate may begin to increase again before the injection is ended. For this reason the table does not always give the lowest rate recorded. With the stronger concentrations the heart did not begin to resume its normal until the injection was finished.

TABLE 1

Showing the influence of adrenalin in small doses on the pulse-rate of nine unanaesthetized dogs. In experiments 7 and 8 crystalline adrenalin was used; in all others adrenalin hydrochlorid. The exponents represent successive injections in the same experiment

EXPERIMENT NO.	PULSE RATE AT BEGINNING	CC. INJECTED	STRENGTH	LENGTH OF INJECTION	PULSE RATE AT END OF INJECTION
1	88	3.5	1: 50,000	3′	64
2	140	3.0	1: 50,000	2′ 30″	65
3^1	93	3.0	1: 50,000	2′ 40″	60
3^2	69	3.0	1: 50,000	2′ 20″	45
3^3	100	3.0	1: 100,000	2′ 10″	90
3^4	129	2.5	1: 100,000	3′	108
3^5	150	3.0	1: 100,000	3′ 05″	141
4^1	124	3.0	1: 100,000	1′ 30″	90
4^2	138	3.0	1: 50,000	1′ 30″	70
4^3	122	3.0	1: 50,000	3′ 55″	90
5^1	72	3.0	1: 50,000	1′ 55″	68
5^2	87	3.0	1: 500,000	1′ 20″	60
5^3	67	2.0	1: 100,000	3′ 45″	67
6^1	99	3.0	1: 100,000	2′ 35″	66
6^2	90	3.0	1: 500,000	1′ 40″	75
6^3	86	2.0	1: 500,000	3′	78
7^1	96	2.5	1: 50,000	2′	66
7^2	90	2.0	1: 200,000	1′ 50″	48
8^1	108	2.0	1: 50,000	1′ 05″	75
8^2	99	2.0	1: 50,000	1′ 55″	72
8^3	117	2.0	1: 200,000	2′ 15″	96
8^4	120	2.0	1: 200,000	1′ 45″	84
9^1	117	2.0	1: 50,000	1′ 35″	66
9^2	123	2.0	1: 50,000	2′ 05″	96
9^3	123	2.0	1: 200,000	2′ 10″	132

The pulse-rate was followed in all cases for from one to four minutes after the injection. In nearly all experiments the heart had reached its former rate or exceeded it by the end of the third or fourth half-minute. In 12 of the 25 separate injections the rate after the injection had been finished rose somewhat above its previous level.

SUMMARY

Intravenous injection of physiological amounts of adrenalin into intact unanaesthetized dogs with good vagal tone invariably causes a decrease in the heart-rate.

The action of the adrenalin is doubtless twofold; it accelerates the heart by direct stimulation and inhibits it reflexly through the vagus. In our experiments the net result of this balanced mechanism was always a decrease in pulse-rate.

For this reason a secretion of adrenalin could hardly play a part in the immediate cardiac acceleration which follows moderate exercise.

In exercise which has been pushed to an extreme degree, however, the accelerating action of adrenalin might well become predominant, for in this condition we have a great decrease in vagal tone. In regard to the heart-rate then adrenalin seems to act just as it does in so many other instances; that is its stimulating effect becomes apparent at a time of great physiological need.

THE INFLUENCE OF THE OIL OF CHENOPODIUM ON THE CIRCULATION AND RESPIRATION

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The frequent occurrence of circulatory and respiratory disturbances observed in the course of studies on the toxicity of the oil of chenopodium (1), (2) suggested the present investigation. The experiments were carried out on different animals, several anesthetics being used for the operative procedures. Morphine-ether anesthesia or chloretone in alcohol was employed in experiments on dogs, ether alone was given to cats, while rabbits were anesthetized with ether or urethane, the latter being given by mouth through a stomach tube.

The oil of chenopodium was introduced in the form of 1 or 2 per cent emulsion, which was made up by adding the oil to 0.9 per cent salt solution, usually but not always containing 5 per cent of acacia, 1 per cent cocoanut oil, and a few drops of sodium carbonate solution. The mixture was then vigorously agitated in a shaking machine until a very fine emulsion resulted. This was injected into the femoral vein from a burette. An emulsion containing all the ingredients in the same proportion, but without chenopodium, was used as a control, and was injected into the femoral vein on the opposite side. It may be stated in advance that blood pressure, after the administration of the control solution, was only slightly increased or not affected at all. No change in respiration was observed at any time. The injections were made from 50 cc. graduated burettes which were held in a Liebig condenser containing water that was maintained at body temperature or a little below by allowing it to circulate through a glass jar, the contents of which were kept warm by means of an electric bulb immersed in the liquid. Blood pressure was recorded by a mercury manometer connected with the carotid artery in the usual fashion, a saturated sodium sulphate solution being used as an anti-coagulating fluid. Respiration was recorded by two receiving tambours, one applied to the thorax

and the other to the abdomen, each of these being connected with a recording tambour by means of rubber tubing. In some experiments the respiration was recorded by a tambour connected with the trachea.

The volume of the kidney was recorded by means of the Roy oncometer in the earlier experiments but air transmission alone was substituted later, the kidney being placed into a covered aluminum box provided with an opening for the renal pedicle. By means of lanolin, the oncometer was made air-tight. One end of a brass tube was fitted into an opening in the top of the oncometer, the other end being connected by means of rubber tubing, with a recording tambour. Variations in volume of the kidney could thus be readily observed.

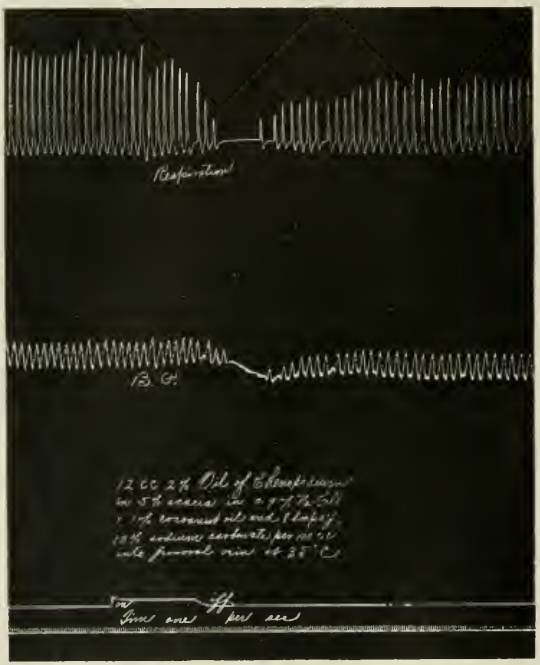


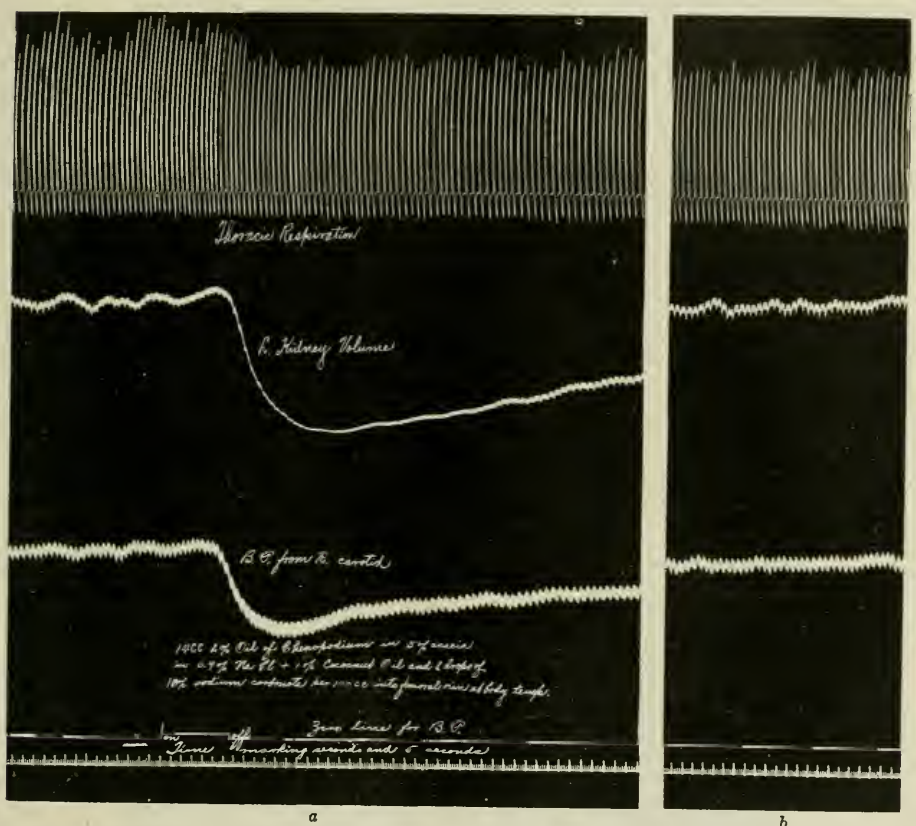
Fig. 1. Dog 218. First injection of 0.02 cc. chenopodium per kilo. Fall of blood pressure, apnoea, and decreased amplitude and rate of respiration.

EXPERIMENTS ON DOGS

Effect on the circulation

A fall of blood pressure, varying in extent and duration was usually observed after the intravenous injection of the oil of chenopodium. In experiments in which morphine-ether was employed, the initial dose of 0.02 cc. per kilo caused a decrease in blood pressure of 10 to 15 mm. Hg or 7.5 to 10 per cent (fig. 1). When a second injection was made, the effects of the same amounts were more pronounced. The fall of blood pressure increased from 7.5 to 12 per cent in one experiment, in another from 10 to 24 per cent, in a third from 9 to 14 per cent, there being little or no difference in the speed of in-

jection. In one experiment, however, the effect was more marked, and was also reversed, the initial injection of the same dose having produced a fall of blood pressure amounting to 35 per cent, while a second injection produced a fall of blood pressure of 16 per cent.



a b

Fig. 2. (*a*) Dog 221. Chloretone—alcohol anesthesia. Marked fall of blood pressure and diminished volume of kidney after the injection of 0.02 cc. chenopodium per kilo. Respiration only slightly depressed. (*b*) Also shows recovery 15 minutes later.

When anesthesia was produced by means of chloretone and alcohol, the initial injection of 0.02 cc. oil of chenopodium per kilo decreased blood pressure 40 to 50 per cent (fig. 2). A second dose, given after an interval of 30 minutes, was followed by the same effect in one experiment, but in two others blood pressure fell considerably less, the

decrease being 20 instead of 50 per cent in one experiment, and about 30 per cent in another in which the initial injection caused a fall of blood pressure of 65 per cent.

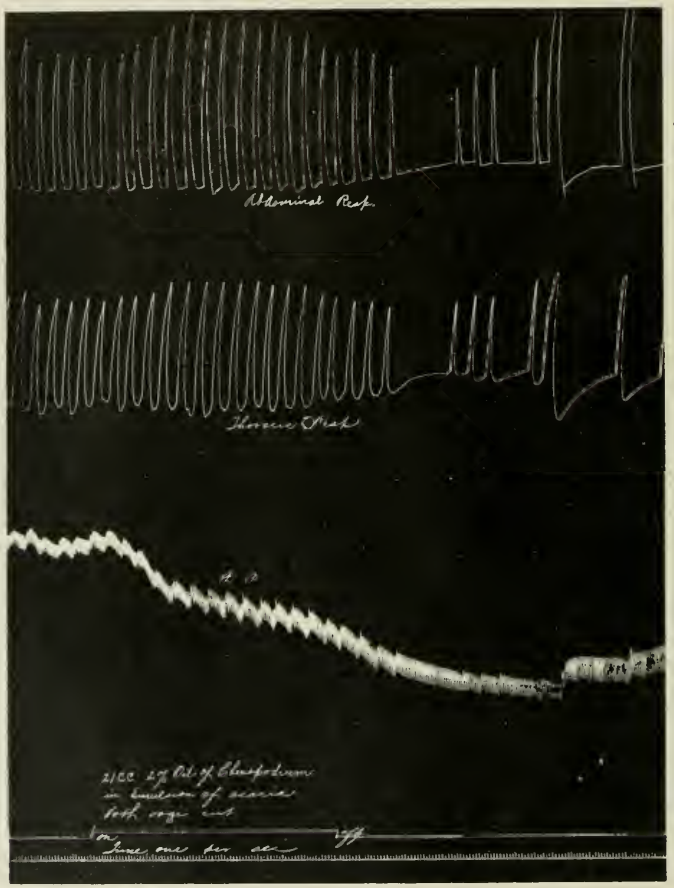


Fig. 3. Dog 210. 0.06 cc. chenopodium per kilo. Showing marked fall of blood pressure and apnoea.

A number of experiments with larger doses have also been carried out. The initial injection of 0.05 and 0.06 cc. oil of chenopodium per kilo produced in dogs, under morphine-ether anesthesia, a fall of blood pressure from 160 mm. to 80 mm., or 50 per cent in one experiment (fig. 3). In another it fell from 155 to 120 mm., or 21 per cent, although

the rate of injection in both was the same, being 1 cc. in about four seconds. In a third subject, likewise under morphine-ether anesthesia, in which the speed of injection was 1 cc. in 16 seconds, 0.05 cc. oil of chenopodium per kilo produced a fall of blood pressure from 115 to 75 mm., or 34 per cent. It is evident, therefore, that the rate of injection does not modify the action of oil of chenopodium on blood pressure. Similar results were obtained with smaller doses in several experiments. The rate of injection is an important factor, however, in determining the character of the fall of blood pressure, which was abrupt when carried out rapidly, but gradual when the speed was reduced.

The effect of repeated injections varied in our experiments, a second dose of the same size, as already noted, producing in some cases an increased fall of blood pressure. In others, on the contrary, this was much less than that caused by the previous initial injection. Subsequent injections decreased the reaction to chenopodium until the response of the circulation completely disappeared (fig. 4) even when larger amounts were introduced. Although the quantity required to produce this effect varied in different individuals, a progressive decrease in the intensity of the reaction was observed until blood pressure was no longer affected by chenopodium. This condition was usually attained when the total amount received exceeded 0.2 cc. per kilo, although in two experiments the depression of the circulation was only slightly affected after doses of 0.105 and 0.12 cc. per kilo had been introduced. The action of repeated injections is illustrated in the following experiments:

In one experiment a dose of 0.085 cc. per kilo produced a maximum fall of blood pressure of 55 per cent. The decrease in the next injection of the same amount was 35 per cent, but after the following injection the fall of blood pressure was only 20 per cent.

In another experiment (fig. 3) the initial injection of 21 cc., or 0.06 cc. per kilo, produced a fall of blood pressure of 50 per cent, and only 7 per cent at the seventh injection when the same dose was given, the total amount previously injected being approximately 0.3 cc. In the same experiment 0.04 cc. per kilo, given in a third injection was followed by a fall of blood pressure of 48 per cent. Such a dose given in the eighth injection had no effect on blood pressure, the rate of injection being practically the same.

In a third experiment 0.02 cc. per kilo, introduced in 15 seconds at the fifth injection, caused a fall of blood pressure, amounting to 20 per

cent. Double this dose per kilo at the tenth and eleventh injections, after a total of 0.236 cc. had been given, failed to produce any change in blood pressure.

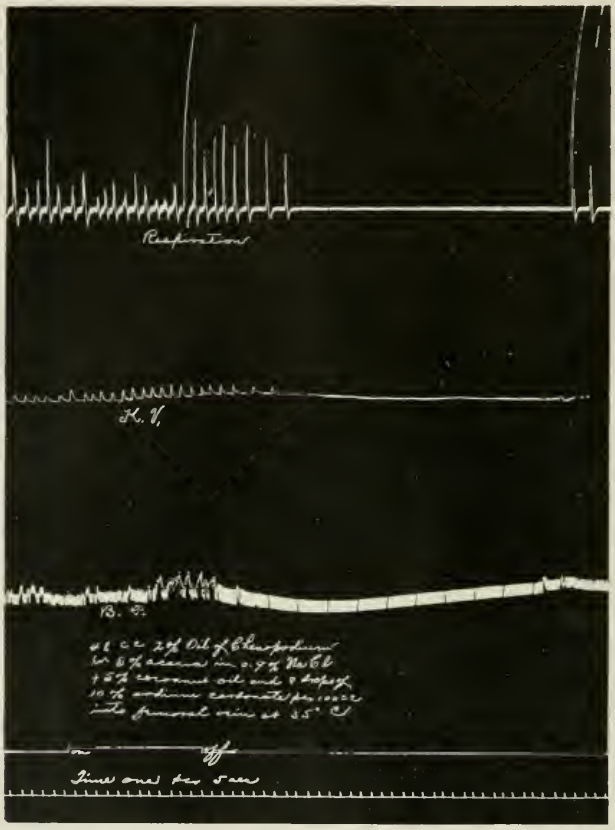


Fig. 4. Dog 219. Prolonged apnoea; $3\frac{3}{4}$ minutes after injection of chenopodium, then dyspnoea. Blood pressure not affected by the injection of 0.08 cc. chenopodium per kilo. Total amount previously injected 0.24 cc. per kilo.

In a fourth experiment 0.04 cc. per kilo, injected in 35 seconds, lowered blood pressure 20 per cent at the sixth injection. A dose of 0.085 cc. per kilo, given at the ninth injection, produced a rise of blood pressure of 11 per cent (which might be expected if the same volume of the control solution were injected). Such a dose injected 34 minutes previously lowered blood pressure 25 per cent.

Similar results were obtained in several other cases. The condition of the blood pressure at the time the failure to respond to oil of chenopodium occurs may appear to be an important factor in the determination of this result, for blood pressure at the time of the injection was considerably lower than at the beginning of the experiment. Analysis shows, however, that this is not the case. In two experiments in which blood pressure fell from 130 to 85 mm. Hg. approximately the same amount of chenopodium produced a fall of blood pressure, in one case 35 per cent, in the other 23.5 per cent. In three subjects in which blood pressure fell from 155–160 mm. Hg. at the beginning of the experiment to about 100 mm. Hg, the reaction to chenopodium was fairly good in two, a fall of blood pressure of 35 per cent being observed in one case after injecting one gram, or 0.085 cc. per kilo. In another 0.06 cc. per kilo produced a fall of blood pressure of 30 per cent. In a third 0.04 lowered blood pressure 10 per cent.

It is evident, therefore, that the reaction to chenopodium may persist though blood pressure had fallen one-third. The experiments carried out under alcohol chloretone anesthesia afford additional evidence that the reaction of the circulation to oil of chenopodium is not determined by the height of the blood pressure, for it will be recalled that small quantities caused a very marked effect when the maximum blood pressure was only 100 mm. Hg, while in many instances in which the height of the blood pressure was 40 to 50 mm. Hg, or even less, the administration of oil of chenopodium produced depression of the circulation to a very considerable degree. Decreased reaction or complete failure of the circulation to react to drugs after repeated injections have been reported by several investigators. Sollmann and Pilcher (3) observed in dogs that large doses of caffeine lowered blood pressure to 50 or 60 mm. Hg, but additional injections failed to cause a further decrease. This is attributed by them to the low blood pressure preceding the injection, since a dose of caffeine which was without effect when blood pressure was under 45 mm. Hg produced a considerable change if injected during periods of high blood pressure. Blackford and Sattford (4) found that the initial dose of an extract of goitre lowers blood pressure in dogs but subsequent injections produced little or no effect. Roth (5) reported similar observations recently with Pituitrin in different animals.

Changes in the volume of the kidney indicate that the fall of blood pressure is of cardiac origin since the oncometric records have almost always varied directly with the changes in blood pressure, being almost

parallel after some injections (fig. 2). In some experiments in which blood pressure no longer reacted to oil of chenopodium, its introduction into the blood stream, especially when large amounts were injected, was followed by an appreciable increase in volume of the kidney, although smaller quantities failed to produce similar changes. That cardiac depression was produced by chenopodium was also indicated by a significant reduction in the rate of heart action, which may be seen on examination of figures 6 and 7. A marked decrease in cardiac vagus irritability was obtained, even after moderate doses were administered. When the vagi were divided after the large amounts of oil of chenopodium were injected, blood pressure rose a few millimeters or remained unchanged.

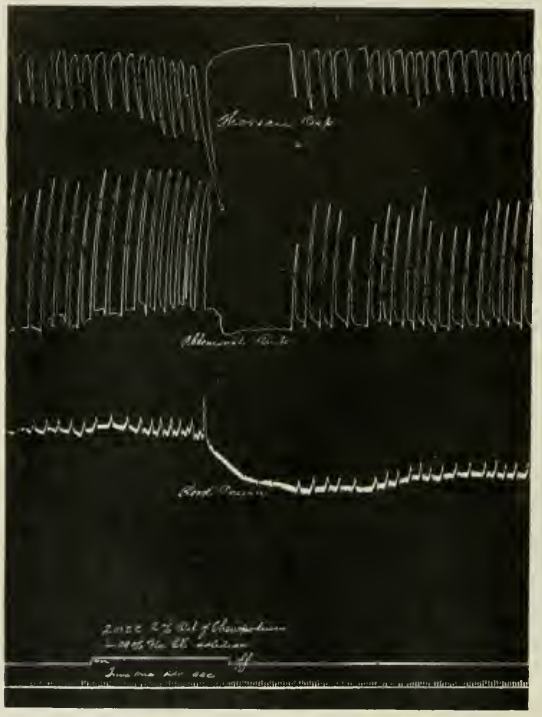


Fig. 5. Dog 209. 0.021 cc. chenopodium per kilo. Apnoea, with decreased respiratory amplitude and fall of blood pressure.

Effect on respiration

The intravenous injection of the oil of chenopodium was likewise followed by depression of respiration. This varied with the size of the dose and sometimes also with the speed with which it was introduced into the blood stream. Small doses, 0.02 to 0.028 cc. oil per kilo of body weight, produced with few exceptions, a decrease in the amplitude, the rate of respiration being affected much less frequently when such amounts were introduced directly into the circulation. In some instances arrest of respiration for a brief period (Dog 209, fig. 5), with prompt recovery was observed. The activity of larger doses was much more marked. The injection of 0.04 cc. oil of chenopodium per

kilo produced slowing of respiration more frequently than with small doses, the amplitude of thoracic respiration being decreased considerably at the same time. The effect on abdominal respiration was less pronounced (fig. 6, Dog 210). Very striking results were noticed in

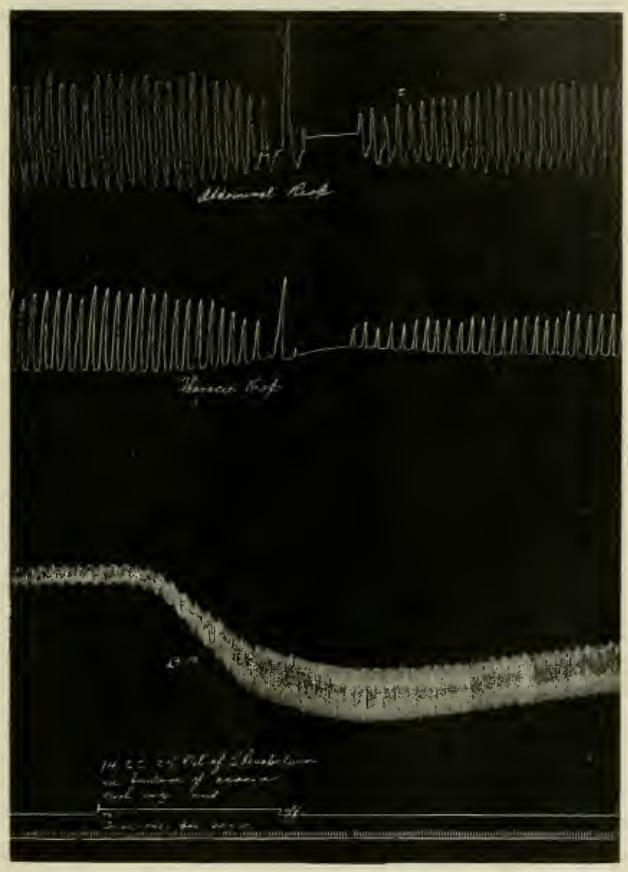


Fig. 6. Dog 210. Apnoea. Respiratory amplitude decreased. Marked fall of blood pressure after the injection of 0.04 cc. chenopodium per kilo.

some experiments (fig. 7), when the doses were still more increased. After the injection of 0.06 cc. oil of chenopodium the rate was very frequently diminished; the amplitude of thoracic respiration also showed considerable reduction, abdominal respiration, however, being much less

affected in this case. The initial injection of such a dose, produced apnoea which lasted nearly 20 seconds. Respiration improved at the end of this time but the rate was only 50 per cent of that preceding the

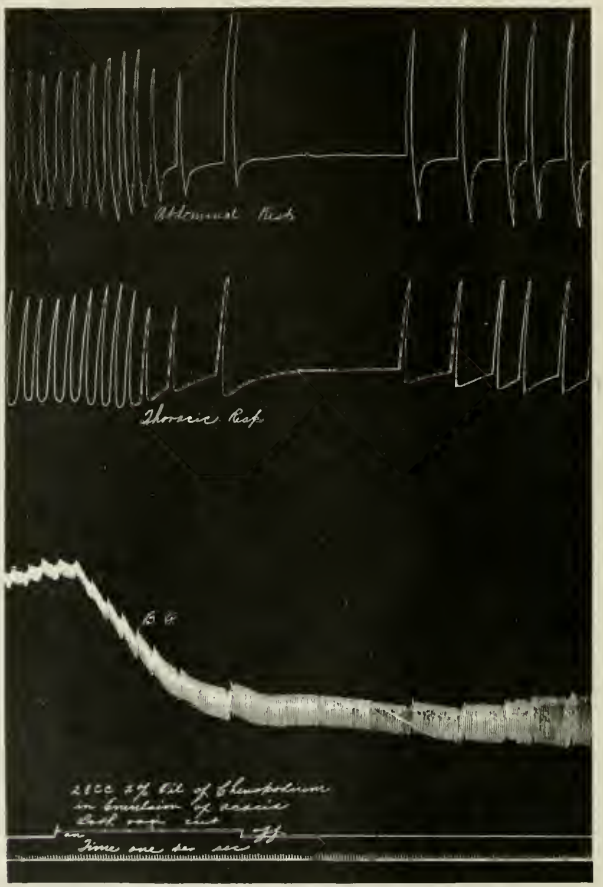


Fig. 7. Dog 210. Showing prolonged apnoea and diminished frequency of respiration after the intravenous injection of 0.085 cc. chenopodium per kilo. Also marked fall of blood pressure and slowing of heart action.

injection. Recovery took place in two minutes. A second injection of 0.085 cc. per kilo (Dog 210, fig. 7), given 16 minutes later caused apnoea which lasted about 50 seconds. In another experiment the initial injection of the same dose was followed by a decrease of frequency of

about 50 per cent. The amplitude of thoracic respiration decreased about 25 to 30 per cent. Abdominal respiration, on the other hand, became more prominent in this case.

The action of repeated injections indicated a tendency to cumulative effect. Even after complete recovery from previous treatment was obtained, another dose of the same size was followed by a more pronounced depression of respiration which was obtained with small, as well as with large doses.

Observations were also made on the effect of the speed of injection. When 0.02 cc. per kilo were introduced at the rate of 15 to 30 cc. per minute the action was practically the same, but the introduction of 0.05 cc. to 0.06 cc. per kilo, given at the rate of 7.5 cc. to 18.0 cc. per minute, showed differences that were very striking. The slower injections of small and of large amounts produced a slight decrease of amplitude of thoracic respiration which was preceded by slight stimulation, but rapid injections were followed in the case of small doses by a marked reduction in rate and decrease in amplitude of thoracic respiration lasting more than $1\frac{1}{2}$ minutes, while large doses were followed by apnoea lasting about a half minute and disturbed rhythm with diminished frequency of respiration from which it recovered in about $2\frac{1}{2}$ minutes.

It will be observed, therefore, that the behavior of the respiratory mechanism towards chenopodium presents important differences from that of the circulation. While the latter frequently showed a tendency towards lessened response after one or two injections of moderate amounts have been made, as already pointed out, the reaction to chenopodium finally disappearing altogether even after large doses have been introduced, respiration, on the contrary, became increasingly slower until it was arrested altogether after the administration of amounts that were much less effective in the earlier stages of the experiment. Blood pressure and cardiac action, which have nearly always been fairly good at the time oil of chenopodium ceased to be effective for the circulation, continued with little or no change for a considerable period of time, while respiration manifested signs of increased disturbance. In some experiments the frequency of the heart beat was still well within the normal rate at this time, the blood pressure being 50 mm. Hg and in some experiments reaching a height of 80 to 90 mm. Hg for about $2\frac{1}{4}$ minutes after respiration had ceased (Dog 219, fig. 4).

Prolonged periods of apnoea were also observed in the final stages

of most of our experiments, usually a short time after the last dose of oil of chenopodium. In one experiment apnoea set in $2\frac{1}{2}$ minutes after the final dose and lasted $3\frac{1}{2}$ minutes. The heart stopped at the end

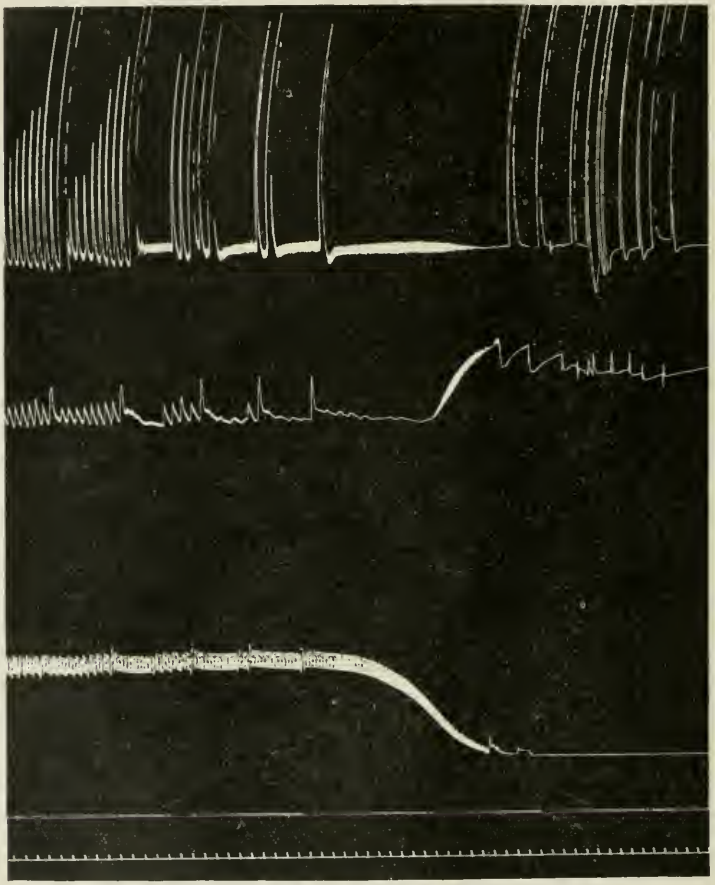


Fig. 8. Dog 220. Apnoea (upper curve). Cardiac paralysis (lower curve) and dyspnoea. Middle curve shows kidney volume. Time, 5 seconds (lowest line).

of this period, apnoea being succeeded by well marked dyspnoea which lasted 2 minutes. In another experiment (fig. 8) 6 minutes after the final dose, which was 0.08 cc. per kilo in this case, several periods of apnoea varying between 20 to 80 seconds were observed, blood pressure during this time was 55 mm. Hg. As blood pressure fell apnoea was

at once followed by dyspnoea and death. This sequence of events, prolonged apnoea, cardiac paralysis, and dyspnoea was observed in most experiments on dogs.

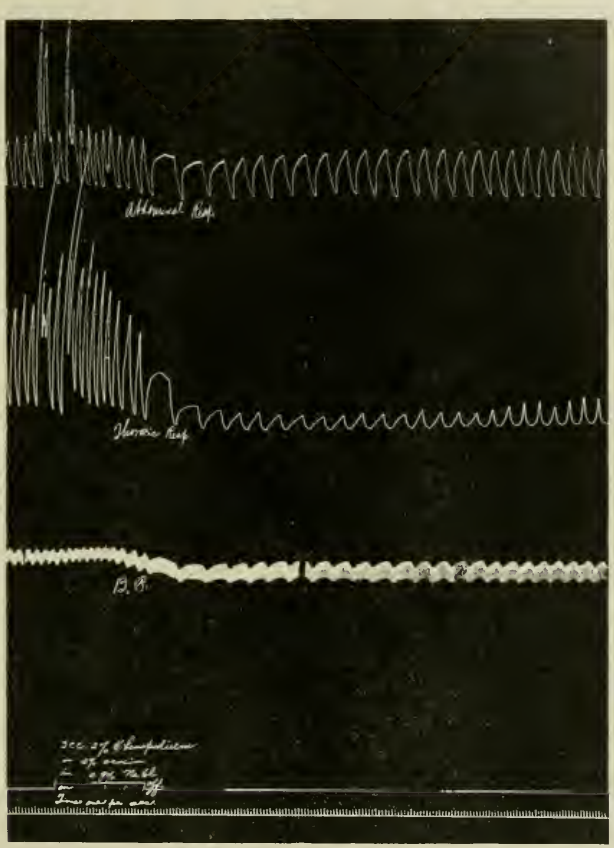


Fig. 9. Cat 287. Intravenous injection of 0.04 cc. chenopodium per kilo. Moderate fall of blood pressure with marked depression of respiration.

EXPERIMENTS ON CATS

The action of the oil of chenopodium when given intravenously was somewhat different in cats. Its effect on respiration was more marked than in dogs. The initial injection of 0.02 to 0.04 cc. per kilo (fig. 9), produced a fall of blood pressure of 6 to 8 per cent, while respiration was depressed to a marked degree, the amplitude, as well as the

rate having been decreased very considerably. A second injection produced a greater effect. Blood pressure fell nearly 10 per cent after smaller doses and 25 per cent after larger doses. Thoracic respiration ceased immediately after injection; abdominal respiration continued, but became much slower. The fall of blood pressure after the initial injection of a dose of 0.06 cc. per kilo was 32 per cent in one case. In another experiment in which 0.085 cc. per kilo was given blood pressure fell 45 per cent.

The increasing effects of oil of chenopodium on the circulation and respiration observed after repeated doses (figs. 10 and 12) failed to manifest themselves, however, in some cases. As shown in Cat No. 320, respiration was not affected either by the sixth or seventh injection, while the blood pressure fell 10 mm. or 11 per cent as a result of the seventh injection.

Cat 287. Weight, 1600 grams. Ether anesthesia

First injection at 2.07 p.m. of 3.2 cc. (0.04 cc. per kilo), 2 per cent emulsion of oil of chenopodium. Injected in 30 seconds. Blood pressure fell from 125 mm. to 115 mm. Hg, or 8 per cent. Recovered in 5 minutes. Abdominal respiration decreased in amplitude fully one-third, while thoracic respiration also became very superficial. The rate decreased from 20 to 6 per minute. Considerable improvement took place during the next 5 minutes, but recovery was incomplete at this time.

Second injection at 2.12 p.m. 3 cc. of 2 per cent emulsion of oil of chenopodium were injected in 60 seconds. Blood pressure fell from 120 to 90 mm. Hg, or 25 per cent, and remained at this level for about 2½ minutes. The rate of cardiac action was also appreciably decreased. Recovered 7 minutes after injection. Thoracic respiration stopped immediately after injection and began to recover slowly two minutes later. Abdominal respiration became slower after injection but improvement began within one and a half minutes.

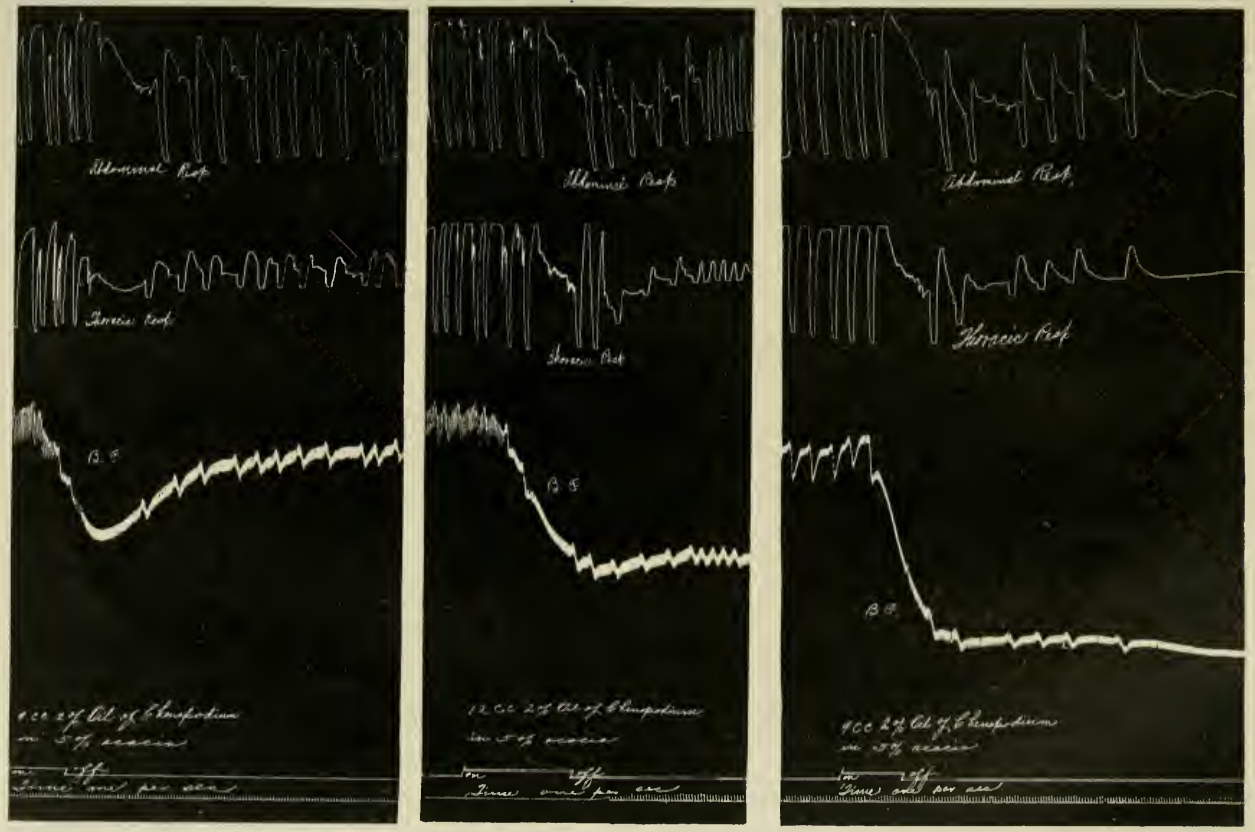
Third injection at 2.19 p.m. Blood pressure 120 mm. Hg. Three cc. of emulsion of oil of chenopodium were injected in 40 seconds. Blood pressure equals 95 mm. Hg. Frequency of respiration decreased 50 per cent, thoracic respiration disappeared. Abdominal respiration markedly decreased. Note cumulative effect on respiration.

Cat 300, female. Weight, 3080 grams. Well fed

December 4, 1914. Ether anesthesia.

1.45 p.m. Received 18 cc. 5 per cent cocoanut oil emulsion in acacia in 2 minutes. Slight rise of blood pressure.

1.58 p.m. Central end of left vagus stimulated for 5 seconds, the right vagus being intact. Distance of P. from S. coil 8 cm. Marked fall of blood pressure and slowing of the heart. Respiration stimulated.



Cat 300. Showing effect on respiration and circulation after the injection of chenopodium.

Fig. 10. 0.06 cc. per kilo.

Fig. 11. 0.08 cc. per kilo, 8 minutes after first injection.

Fig. 12. 0.06 cc. per kilo, 23 minutes after second injection.

2.07 p.m. Central end of left vagus stimulated. Distance P. from S. coil 16 cm. Slight fall of blood pressure. Respiration slowed.

2.08 p.m. 9 cc. 2 per cent oil of chenopodium given in 5 per cent emulsion of acacia. Blood pressure fell from 185 to 125 mm., or 32 per cent. Respiration stopped for about 20 seconds, then returned, but was much slower than before. Amplitude of thoracic respiration was much more depressed than abdominal.

2.12 p.m. Blood pressure recovered. Central end of vagus stimulated 5 seconds. Distance of P. from S. coil 8 cm. No effect on blood pressure but respiration distinctly stimulated.

2.16 p.m. 12 cc. 2 per cent emulsion oil of chenopodium given in 5 per cent acacia and cocoanut oil. Blood pressure fell from 190 to 110 mm., or about 42 per cent. Respiration stopped for about 20 seconds then returned but was much slower. Thoracic respiration very feeble, abdominal respiration slower than before injection but depth not markedly less than before. While blood pressure remained low, 18 cc. of an emulsion of 5 per cent cocoanut oil in 5 per cent acacia were injected at the rate of 12 cc. per minute. Blood pressure rose gradually from 105 to 140 mm. in 40 seconds but there was no noticeable improvement in respiration. Respiration, on the contrary, became much slower. Almost immediately after injection of cocoanut oil emulsion, blood pressure fell about 40 per cent; respiration became irregular, then slow. Another injection of 12 cc. 5 per cent cocoanut oil emulsion was made in 70 seconds; blood pressure steadily rose from 60 mm. to 150 mm. Hg in half a minute. Respiration became deeper and more frequent toward the end of the injection, but this lasted half a minute and stopped altogether.

2.39 p.m., or 16 minutes later, the injection of 9 cc. of 2 per cent oil of chenopodium in 20 seconds caused a sudden drop of blood pressure from 170 to 70 mm., respiration becoming slow.

2.41 p.m. No improvement in blood pressure. Respiration became slower than before. Eighteen cc. cocoanut oil were injected; blood pressure became still slower, respiration stimulated at first, then stopped. Animal dead.

Cat 286. Weight, 2740 grams

1.43 p.m. 12 cc. (0.087 cc. per kilo) emulsion of 2 per cent oil of chenopodium injected in 70 seconds. Blood pressure fell from 145 to 80 mm. Hg, or 45 per cent, by the time the injection was completed. Two minutes later no change in blood pressure. Respiration much slower, thoracic respiration became very superficial, abdominal respiration deeper, but much slower than before injection. Ten minutes later blood pressure was 130 mm. Hg. Respiration was much improved but did not return to normal.

Second injection was made of 12 cc. 2 per cent emulsion of oil of chenopodium. Blood pressure fell from 130 to 60 mm. Hg by the end of the injection, 100 seconds. Effect on respiration was very marked; abdominal respiration less depressed than thoracic. Forty-five seconds after injection respiration stopped, blood pressure was but a few mm. Hg, heart continued to beat 40 seconds after respiration stopped.

Cat 320. Weight, 3.3 kilos

3.15 p.m. Injected 7 cc. 1 per cent oil of chenopodium in 50 seconds. Blood pressure before injection was 160 mm. after injection 150 mm. Respiration became much slower, amplitude was decreased.

3.19 p.m. Blood pressure was 160 mm. Hg. Injected 7 cc. oil of chenopodium in 53 seconds. Blood pressure was 145. Blood pressure began to rise at once; at 3.23 p.m. the blood pressure was 160 mm. Hg. Injected 15 cc. 1 per cent oil of chenopodium in 5 minutes.

3.28 p.m. Blood pressure was 140 mm. Hg. Recovered in 3 minutes.

3.31 p.m. Blood pressure was 160 mm. Hg.

3.33 p.m. Injected 13 cc. 1 per cent oil of chenopodium in $1\frac{3}{4}$ minutes. Blood pressure before injection was 155 mm. Hg. At the end of the injection blood pressure was 125 mm. No change in 3 minutes, then rose gradually. Respiration decreased in force and frequency.

3.49 p.m. Blood pressure was 145 mm. Hg. 13 cc. 1 per cent oil of chenopodium injected in 72 seconds. Blood pressure before injection was 145 mm. Hg at the end of the injection 100 mm. Hg, which continued without change 6 minutes before recovery began. Respiration was much slower and weaker. Thoracic respiration just perceptible; abdominal respiration regular and fairly strong, but weaker than before injection.

4.15 p.m. Blood pressure 110 mm. Hg. Injected 13 cc. 1 per cent oil of chenopodium in $3\frac{1}{2}$ minutes. Blood pressure 80 mm. Hg. Respiration remained unchanged.

4.28 p.m. Blood pressure 90 mm. Hg, injected 13 cc. 1 per cent oil of chenopodium in $1\frac{1}{2}$ minutes. Blood pressure 80 mm. Hg. No change in respiration.

4.34 p.m. Blood pressure 70 mm. Hg. Injected 30 cc. 1 per cent oil of chenopodium in $3\frac{1}{2}$ minutes, At the end of injection blood pressure began to decline rapidly and reached zero in about one minute. Respiration stopped before heart action was arrested. A few respiratory movements noticed after heart stopped.

EXPERIMENTS ON RABBITS.

The circulatory changes produced by chenopodium as observed in rabbits, under ether or urethane anesthesia, indicate that even a small dose may produce considerable depression. Blood pressure fell 16 to 20 per cent after initial doses of 0.022 to 0.027 cc. oil of chenopodium per kilo were injected intravenously (fig. 13). The rate of injection was about 7 cc. per minute, in some experiments, while the time occupied in other experiments was about 5 seconds for the introduction of the entire amount. Te fall of blood pressure was usually prompt, but it began to rise almost immediately, often within 30 seconds from the time the lowest level was attained. Recovery was accomplished in some experiments in 3 to 5 minutes, in others the process occupied more than double this time. Larger doses may be more active though this was not always the case. In two experiments with 0.04 and 0.08 cc. oil of chenopodium per kilo the effect of the initial injection did not

Fig. 13. Rabbit 1763. Showing fall of blood pressure and stimulation of respiration after 0.02 cc. chenopodium per kilo.

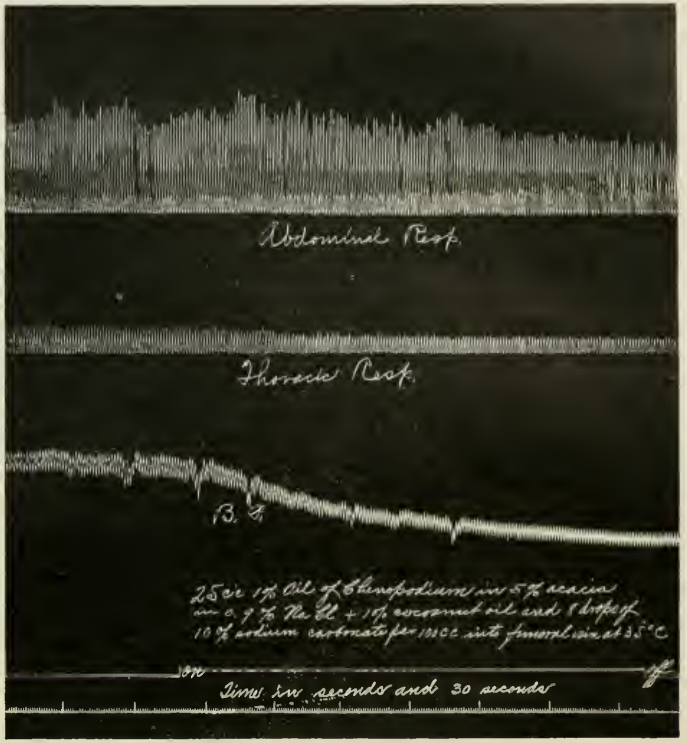


Fig. 14. Rabbit 1772. The intravenous injection of 0.08 cc. chenopodium per kilo shows marked fall of blood pressure and moderate decrease of respiratory amplitude.

differ from that of smaller doses. But this is in all probability exceptional as the results were different when the experiments were repeated in other rabbits. The initial injection of 0.08 cc. of oil of chenopodium per kilo (fig. 14) produced a fall of blood pressure amounting to 45 mm. Hg or 39 per cent in one experiment. The relative effect of subsequent injections did not differ appreciably from that produced by the initial dose. This was observed after experiments with small as well as with larger doses, and may be readily seen from the following abbreviated protocols.

Rabbit 1772. Gray female. Weight 3.19 kilos.
March 8, 1915. Received 6 grams urethane by mouth through stomach tube.

	EMULSION OF 1 PER CENT CHENOPODIUM INJECTED	BLOOD PRESSURE		FALL OF BLOOD PRESSURE		DURATION OF INJECTION
		Before injection	After injection	mm. Hg	Per cent	
	cc.					
1.08	25.0	115	70	45	39	3 min. 20 sec.
1.41	25.0	90	58	32	35	4 min. 5 sec.
2.19	25.0	65	35	30	46	2 min. 40 sec.
2.54	20.0					2 min. 50 sec.

NOTE: Blood pressure gradually fell and rabbit died at 3.01 p.m.

Rabbit 1763. Gray male. Weight 2.3 kilos.
March 3, 10.30. Received by mouth through stomach tube, 2.5 grams urethane.

12.01	4.6	95	75	20	21	20 sec.
12.15	5.0	105	80	25	24	36 sec.
12.30	5.0	102	85	17	16	43 sec.
12.40	5.0	100	85	15	15	90 sec.
12.46	10.0	100	70	30	30	70 sec.

Rabbit 1718. Weight 2.2 kilos. Ether anesthesia.
6 cc. control solution injected—no effect.

	EMULSION OF 1 PER CENT CHENOPODIUM INJECTED	BLOOD PRESSURE		FALL OF BLOOD PRESSURE		DURATION OF INJECTION
		Before injection	After injection	mm. Hg	Per cent	
	cc.					
1.55	6.0	118	95	23	20	55 sec.
2.03	6.0	110	84	26	23.6	
2.19	5.5	125	96	29	23+	1 min.
2.30	5.0	106	94	12	11+	1 min.
2.48	6.0	85	65	20	23+	75 sec.

A progressive increase in the fall of blood pressure was observed, however, in some experiments after chenopodium. A second injection of the same amount as the first was twice as effective, while a third injection was approximately three times as active as the initial dose, the amounts introduced each time being the same. The absolute and relative fall of blood pressure are shown in the following table:

	1696 0.08 cc.	1660 0.02 cc.	1702 0.027 cc.	1661 0.04 cc.
1	20 mm. or 23 per cent	25 mm. or 18 per cent	15 mm. or 16 per cent	22 mm. or 20 per cent
2	32 mm. or 46 per cent	45 mm. or 37 per cent	30 mm. or 36 per cent	28 mm. or 28 per cent
3		50 mm. or 50 per cent	40 mm. or 50 per cent	35 mm. or 35 per cent
Remarks	Died after receiving 0.16 per kilo.	Died after receiving 0.083 per kilo.	Died after receiving 0.08 cc. per kilo.	Died after receiving 0.24 cc. per kilo.

It may be remarked that the fatal dose in the experiments presented in this table was considerably smaller than in those rabbits in which no cumulative effect on the circulation could be demonstrated. The acute fatal dose of chenopodium when administered intravenously varied in our experiments between 0.3 and 0.35 cc. per kilo and in one instance it was 0.48 cc. per kilo. The results obtained in Rabbit 1661 in which the effect of repeated injections were much less marked than in the other three rabbits given in the table suggest that the resistance to chenopodium might be a factor in determining cumulation. It may be of interest in this connection to call attention to the condition of the heart in Rabbit 1660. Post mortem examination indicated the presence of serious damage to the myocardium, brought about possibly by repeated bleeding extending over a period of several weeks which were made by thrusting a hypodermic needle into the organ. Although this case does not afford sufficient evidence for any positive statement regarding the relation of the condition of the heart to circulatory disturbance caused by chenopodium, it is nevertheless suggestive and may perhaps account for similar effects observed in other experiments.

As shown in the experiments cited below, cumulation was likewise indicated when larger doses of chenopodium were injected after considerable quantities had previously been introduced into the circulation. Perhaps also in these cases the increased action was due to damage of the heart caused by the previous treatment with chenopodium.

Rabbit 1709. Weight, 2275 grams

No previous injection.
35 cc. 1 per cent chenopodium injected in 8 minutes.
Blood pressure fell from 100 to 80 mm. Hg, or 20 per cent.

Rabbit 1681. White male. Weight, 2250 grams.

Total quantity previously injected 0.25 cc.
Seven minutes later 22.5 cc. 1 per cent chenopodium injected in 15 minutes.
Blood pressure fell from 88 mm. before injection to 40 mm. or 54 per cent when injection was discontinued.

Rabbit 1718. Weight, 2.2 kilos.

Total quantity of chenopodium injected 0.4 cc, or 0.18 cc. per kilo; 5 minutes after last injection 22.5 cc. 1 per cent emulsion of oil of chenopodium injected in 8½ minutes.
Blood pressure fell from 85 mm. to 40 mm. Hg, or 52 per cent.

The action of chenopodium on respiration was in several respects different from its effects on the circulation. The initial injection of small and moderate doses, the duration of which varied from 5 to 60 seconds, did not produce any respiratory changes in most experiments. In some cases slight, in others transitory but well marked stimulation was observed, even when the initial dose was large (fig. 13). A dose of 0.08 cc. per kilo produced in one rabbit (1772, fig. 14) a moderate decrease of amplitude, while in another it caused a slight depression only. The rate of injection in this case was 4.5 cc. 1 per cent emulsion of oil of chenopodium per minute. Subsequent injections of large doses were much more active, but the case was different when smaller amounts were administered. A second or third injection of a small dose which was not previously effective sometimes produced respiratory depression but this was not always the case. In one experiment a number of injections were made without affecting respiration; only after the fifth injection when 0.05 cc. per kilo at one dose was given the total amount previously received being 0.1 cc. per kilo, was any effect noticed in this experiment, thoracic respiration being markedly depressed but abdominal respiration remained unchanged. Similar results were obtained in another experiment (1681). In this case two doses, each of 0.022 cc. per kilo, produced a slight increase of amplitude. In the third the amplitude of thoracic respiration decreased 33 per cent after a dose of 0.066 cc. per kilo. No change in abdominal respiration was observed. Another injection of 0.1 cc. per kilo begun 7 minutes later and carried out in 15 minutes, produced a still greater

effect on thoracic respiration, the amplitude being decreased 65 per cent, but abdominal respiration remained unchanged. In neither test was the rate affected.

Although the amount required to depress respiration varied a good deal in different individuals, it may be safely stated that when a sufficient amount was introduced into the circulation, respiration became decidedly weaker, amplitude, as well as the rate being decreased. It may be pointed out, however, that the frequency of respiration was but moderately depressed. This was also observed in rabbits in which stimulation occurred at first. Although respiration seemed to be less affected by chenopodium than the circulation, arrest of respiration usually took place either at the same time or before heart action ceased. In several experiments in which the heart was exposed it was found beating feebly and continued some time in this condition—15 minutes to one hour.

DISCUSSION.

A survey of the results obtained in the foregoing experiments shows that the effect of oil of chenopodium on different animals is essentially the same notwithstanding the occasional departure from the general type of action and the marked quantitative differences produced in the same animals under changed conditions of experimentation. This was illustrated by the characteristic action on the circulation and respiration and also by the resistance to chenopodium as indicated by the amounts tolerated, which varied in rabbits between 0.15 cc. and 0.48 cc per kilo, being in most cases between 0.3 cc. and 0.35 cc. While the largest amounts in dogs were 0.47 cc., the smallest was 0.3 per cc. per kilo. Cats succumbed when the total quantities reached 0.2 to 0.36 cc. per kilo. The average is, therefore, approximately the same in all the animals examined. Intravenous injections were usually followed by circulatory and respiratory disturbances. The initial dose produced a fall of blood pressure in dogs, cats, and rabbits but the action when this was repeated was greater in the dog and rabbit than in the cat. The effect on dogs was markedly enhanced, the fall of blood pressure being several times greater when chloretone anesthesia was substituted for morphine and ether. The action in different animals varied still more when the injections were repeated. Thus the failure of the circulation to react after a large amount of chenopodium has been introduced in divided dose, which was frequently observed in dogs, was seldom seen in the cat and was absent in rabbits.

That chenopodium is a respiratory depressant was abundantly shown in our experiments but the behavior of different animals in this respect likewise presented interesting differences. Respiratory depression after the same amounts in proportion to body weight was more marked in cats than in dogs. In rabbits, it will be recalled, small doses may produce stimulation at first, depression having been noticed only after the dose was repeated several times. Besides, apnoea was seldom observed in rabbits even after large doses. Bruning's (6) experiments on the behavior of the red blood cells toward chenopodium are of interest in this connection since he found that hemolysis and methemoglobin may be produced in vitro. It is conceivable that disturbance of respiration may be caused by the hemolytic action and the formation of methemoglobin. We have, therefore, carried out a number of observations with the oil of chenopodium to determine whether methemoglobin and hemolysis may be produced in vivo. Doses of 0.02 cc. to 0.24 cc. per kilo were injected intravenously. A spectroscopic examination of the blood obtained from animals thus treated failed to indicate the presence of methemoglobin. Hemolysis was also absent even after large amounts were administered. This was studied in cats after the introduction of two grams per kilo of the oil into the stomach or small intestine, and in rabbits which received 0.1 to 0.16 cc. per kilo intravenously. A moderate degree of hemolytic action was present in dogs' blood taken after oil of chenopodium was injected intravenously and kept at low temperature for 24 hours, but this was about the same in degree which may be observed when blood is obtained from anesthetized dogs that have not received oil of chenopodium. Depression of the circulation may also be thought of as a factor in determining the respiratory changes observed, but the results obtained in a large proportion of our experiments do not support this view. As shown in figure 2, blood pressure fell about 40 per cent but respiration was only slightly depressed. On the other hand, respiratory depression was observed when the circulation no longer reacted to chenopodium. Moreover, in experiments on rabbits a fall of blood pressure and a coincident stimulation of respiration often occurred.

Paralysis or depression of the vagus may also be thought of in this connection as a possible cause, but some of our observations with chenopodium were made on dogs and cats in which both vagi were cut. As the characteristic effect on respiration was present in both these animals it would seem that the action was central. It is evident that no satisfactory explanation of the respiratory effect can be offered at

present. The work of Loevenhart and Grove (7) is, however, suggestive. After the intravenous injection of the salts of iodoso and iodoxy benzoic acid, the effects on respiration which these investigators observed were strikingly similar to those of chenopodium. They believed that the apnoea was due to the liberation of oxygen in the blood by these substances. As shown by Nelson (8) and later by Wallach (9), ascaridole, which is the active principle in the oil of chenopodium, is a peroxide. Although it does not oxidize guaiac, it is quite possible that its action in vivo may be similar to that of the salts of benzoic acid. The depression of respiration and apnoea may be due, therefore, to the oxygen liberated by ascaridole. This explanation does not seem to apply to the action of oil of chenopodium in the rabbit, but may, indeed, contradict it. That this is not the case, however, appears when the relative amounts of carbon dioxide and oxygen in the blood of different animals, which formed the subjects of our experiments, are taken into consideration. According to Heinz (10), the carbon dioxide, as compared with the oxygen, in the blood of the rabbit, is relatively greater than in the dog or cat. Since carbon dioxide stimulates respiration, according to Henderson (11) and others, its action would be antagonistic to that of oil of chenopodium, thus tending to neutralize the effect produced by the liberation of oxygen. The relatively greater amount of carbon dioxide in the blood of the rabbit would, therefore, prevent the onset of respiratory depression and apnoea. This may also explain the difference in the final stages of intoxication. The simultaneous failure of the circulation and respiration frequently observed in chenopodium poisoning in the rabbit is probably due to direct action on the heart, since respiration is impaired but not abolished. Cardiac asphyxia (Henderson, l. c.), such as would occur in the dog or cat on account of prolonged apnoea is not probable in the rabbit, there being enough oxygen to sustain heart action while the respiration goes on, though very much diminished. Finally the anesthesia produced by chenopodium may also be considered as a factor in the production of apnoea and respiratory depression. According to Henderson the respiratory center is much less sensitive to carbon dioxide in profound anesthesia, but the evidence obtained does not favor this explanation as in profound anesthesia, such as was produced by chloretone and alcohol, the administration of chenopodium, according to this explanation, should have produced apnoea much more readily than when the same was given in morphine-ether anesthesia.

SUMMARY

1. The intravenous injection of doses of 0.02 to 0.085 cc. of chenopodium per kilo produced a fall of blood pressure in dogs, cats and rabbits. Recovery was observed.

2. The effect was greater in dogs than in rabbits or cats.

3. A second injection of the same dose produced a greater effect, but when this injection was repeated until the total amount reached about 0.2 cc. per kilo, no response of the circulation could be observed. This was especially the case in dogs, but to a much smaller extent in cats. This phenomenon was absent in rabbits.

4. Fall of blood pressure was of cardiac origin, as the volume of the kidney decreased with the fall of blood pressure.

5. Frequency of heart action was diminished after oil of chenopodium.

6. A very marked decrease of vagus irritability was observed after oil of chenopodium.

7. Respiratory depression such as decreased amplitude and rate, with apnoea, was also caused by chenopodium, but the effect with small doses was less constant than on the circulation.

8. Cats react more readily than dogs.

9. Small doses may stimulate respiration in rabbits. Apnoea was very seldom observed in the rabbit, even after large doses.

10. No methemoglobin or hemolysis was observed even after the intravenous injection of 0.02 to 0.024 cc. per kilo, or the introduction of two grams per kilo into the stomach or small intestine of the cat.

11. Liberation of oxygen in the body by ascaridole is suggested as a possible cause of respiratory depression and apnoea.

12. Action of chenopodium on respiration is independent of its effect on the circulation.

13. Reduction of sensitiveness of respiratory center to carbon dioxide is not the cause of action of chenopodium on respiration.

14. Amounts of chenopodium tolerated by intravenous injection varied in the same animals. The average is approximately 0.03 to 0.35 cc. per kilo in dog, cat and rabbit.

15. The less depressant action of chenopodium on respiration in the rabbit is attributed to relatively larger amounts of carbon dioxide in the blood.

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INFLUENCE OF DIIODOTYROSINE AND IODOTHYRINE ON THE SECRETION OF CEREBROSPINAL FLUID

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In a previous communication we have shown that thyroid extract slows the rate of secretion of cerebrospinal fluid. The present work was undertaken to discover, if possible, what constituent of the thyroid gland possesses the power of inhibiting the cerebrospinal fluid secretion. In this paper we present the results of experiments with iodothyrine, a commercial derivative of the thyroid gland, and of diiodotyrosine, a synthetic substance closely related to the iodine complex of the thyroid gland. The diiodotyrosine was prepared and kindly furnished for these experiments by Dr. Treat B. Johnson of Yale University.

The anesthesia, rate of secretion of cerebrospinal fluid, and the blood pressure records were obtained with the technique described in a previous paper (1).

Diiodotyrosine. The injection of solutions of this substance into the femoral vein causes no change in blood pressure or in respirations, so that all changes in the outflow of cerebrospinal fluid which result from the injection may be considered as due to the effect of the substance on the secretion of the choroid plexus.

The results of four experiments with diiodotyrosine are given in the table on next page.

Experiment I. The injection of 0.1 gm. of diiodotyrosine intravenously causes a marked decrease in the flow of cerebrospinal fluid. This is pronounced even in the first half hour after injection, the flow dropping from the normal 3.78 cc. to 1.28 cc., a decrease in rate of secretion of 0.0833 cc. per minute. The flow of cerebrospinal fluid gradually grows less to the end of the experiment, two and one-half hours after the injection, although slightly more fluid escapes in the last period than in the two preceding it. The average flow for the five 30-minute periods after injection is only 0.8 cc., a decrease from the normal flow of 2.98 cc. The normal rate of secretion is 0.126 cc. per minute, while

the average rate for the five periods is 0.026 cc. per minute, a decrease in rate of secretion of cerebrospinal fluid after the injection of 0.1 gm. of diiodotyrosine of 0.1 cc. per minute.

Experiment II. The injection of 0.2 gm. of diiodotyrosine in this instance does not show as pronounced an effect on the amount of secretion in the first half hour as was noted with 0.1 gm. in Experiment I. The flow is lessened, however, and steadily decreases after the second period. The flow for the first half hour is 1.28 cc. against 1.59 cc. for the normal period. This is a decrease in rate of secretion from 0.053 cc. per minute to 0.042 cc. per minute. The flow for the second period

Diiodotyrosine

PERIODS	FLOW IN CC. OF C. S. F. FOR 30-MINUTE PERIODS			
	Experiment I. 0.1 gm. injec.	Experiment II. 0.2 gm. injec.	Experiment III. 0.2 gm. injec.	Experiment IV. 0.1 gm. injec.
Before injection	3.78	1.59	2.82	1.62
After injection 1	1.28	1.28	1.63	1.28
2	0.99	1.39	0.66	1.29
3	0.56	1.23	0.77	1.23
4	0.51	0.88	0.78	0.88
5	0.67	0.47	0.28	0.47
6		0.11		0.11
7		0.26		0.26
Average flow for 30 minutes.	0.80	0.80	0.82	0.78
Decrease from normal	2.98	0.79	2.00	0.84

is larger than that for the first, but decreases below the first in the third period.

The flow then rapidly lessens till in the sixth period only 0.11 cc. is secreted an average rate of 0.003 cc. per minute. The flow in the seventh period is somewhat more rapid than in the preceding 30 minutes, but still has a rate of only 0.008 cc. per minute. The average flow for 30 minutes for the seven periods (three and one-half hours) is 0.8 cc. a decrease of 0.79 cc. from the normal flow. This is a decrease in secretory rate of 0.027 cc. per minute.

Experiment III. The injection of 0.2 gm. of diiodotyrosine in another dog gives more pronounced results than in Experiment II. In this experiment the reduction in flow is pronounced in the first half hour with a further marked drop in the second period, while in the third and fourth the flow is practically uniform. The decrease in

flow for the first period is 1.19 cc., a reduction of 0.04 cc. per minute in rate of outflow. The second period shows a decrease from the first period of 0.97 cc. or a reduction in rate of outflow of 0.032 cc. per minute. The third and fourth periods have a slightly greater flow than the second, 0.77 cc. and 0.78 cc. respectively against 0.66 cc. for the second. The fifth period, however, is much slower, only 0.28 cc. escaping in the 30 minutes. The average 30-minute flow for the five periods is 0.82 cc., a decrease from the normal of 2 cc. or a reduction in rate of 0.067 cc. per minute.

Experiment IV. The injection of 0.1 gm. of diiodotyrosine in this experiment gives less striking results than in Experiment I. In fact the results are very similar to those of Experiment II in which twice the amount was injected. The decrease in flow is noticeable in the first half-hour and remains practically the same for the first hour and a half after injection. It then rapidly decreases for one and a half hours, with a slight increase in the last half-hour. The flow in the first period decreases 0.34 cc. from the normal, or a decrease in rate of secretion of 0.011 cc. per minute. The flow is then practically unchanged until the fourth period when 0.88 cc. escapes in 30 minutes. The fifth and sixth periods show reductions to 0.47 cc. and 0.11 cc. respectively. The seventh or final period shows a slight increase to 0.26 cc. The average flow for 30 minutes for the seven periods is 0.78 cc., a decrease from the normal amount of 0.84 cc. or a decrease in rate of secretion of 0.028 cc. per minute.

It is evident from the above experiments that diiodotyrosine in solution when injected intravenously has an inhibitory action on the secretion of the choroid plexus very similar to that of saline extracts of the thyroid gland. The results on different dogs are not uniform, but seem to depend somewhat upon the normal rate of cerebrospinal fluid secretion.

Iodothyrine. The intravenous injections of iodothyrine solution produce no changes in blood pressure or respiration. The results of four injections are given in the table on next page.

Experiment I. The injection of 0.3 gm. iodothyrine gives a decrease of 0.32 cc. cerebrospinal fluid for the first half hour, a decrease in rate of 0.11 cc. per minute. The flow shows a steady reduction which in the fifth and sixth periods has decreased to 0.4 cc., a decrease from normal of 0.95 cc. The average 30-minute flow for the six periods is 0.66 cc. a drop from normal of 0.69 cc. or a decrease of 0.023 cc. per minute in rate of secretion.

Experiment II. The injection of 0.05 gm. iodothyrine has very little influence on the flow of cerebrospinal fluid.

The flow in the half hour before injection is 1.41 cc. and in the first period after injection is 1.10 cc., a reduction of 0.31 cc. or a decrease in rate of 0.11 cc. per minute. However, in the second period the flow returns to normal. In the succeeding two periods it again decreases, the average 30-minute flow for the four periods after injection is 1.15 cc., a decrease of only 0.26 cc. or in rate of only 0.009 cc. per minute. This is almost within the range of the normal decrease.

Experiment III. In this experiment 0.5 gm. iodothyrine gives very little change in the first half hour, the decrease only amounting to 0.09

Iodothyrine

PERIODS	FLOW IN CC. OF C. S. F. FOR 30-MINUTE PERIODS			
	Experiment I. 0.3 gm. injec.	Experiment II. 0.05 gm. injec.	Experiment III. 0.5 gm. injec.	Experiment IV. 0.3 gm. injec.
Before injection	1.35	1.41	1.74	2.97
After injection 1	1.03	1.10	1.65	1.41
2	0.92	1.40	1.17*	0.57
3	0.73	1.23	0.63	0.11
4	0.53	0.90		
5	0.40			
6	0.40			
Average flow for 30 minutes	0.66	1.15	1.15	0.69
Decrease from normal	0.69	0.26	0.59	2.28

* 1.5 gm. injected.

cc., a second injection (1.5 gm.) is then given and the next period shows a more marked reduction. This response reduced the flow 0.57 cc. below the normal. The third period shows a further reduction from normal of 1.11 cc. Owing to an unavoidable accident the experiment was terminated at this point. The average 30-minute flow for the periods after injection is 1.15 cc., a decrease of 0.59 cc. or a drop from the normal rate of 0.058 cc. per minute to 0.038 cc. per minute.

Experiment IV. The injection of 0.3 gm. iodothyrine in another dog gives a more marked decrease than in any of the three previous experiments. The reduction in the first half hour after injection is pronounced, slightly less than half the normal amount of fluid flowing from the cannula. The actual decrease in this period is 1.56 cc. The flow in the third period is slightly larger than in the second, while in

the third it is again greatly reduced. The average flow for a 30-minute period after injection is 0.69 cc., a drop below the normal of 2.28 cc. or a decrease in secretion rate from 0.099 cc. per minute to 0.023 cc. per minute, a reduction of 0.076 cc. per minute.

The experiments with iodothyrine tend to show that small amounts, such as 0.05 gm. have very little influence on the rate of secretion of cerebrospinal fluid. Larger amounts result in a slower rate, but with the exception of Experiment IV, the decrease is not as marked as that produced by diiodotyrosine or saline extracts of thyroid gland.

It is evident from the experiments, both with diiodotyrosine and iodothyrine, that different dogs react very differently to the same amount of substance. As a general rule the most marked responses are in those animals which have normally a very rapid secretion. Sex and age gave apparently no influence, while the weight of the animal seems to make very little difference.

Control experiments have shown that the decrease in flow noted in the above experiments is due to the action on the choroid plexus of the substance injected. A decrease does occur normally, but this is so slight as to be negligible in the two to four hours which are considered in these experiments.

CONCLUSIONS

1. The intravenous injection of diiodotyrosine has an inhibitory influence on the rate of secretion of the choroid plexus. This decrease in rate usually appears in the first half hour after injection, but is not so marked as that obtained with saline extracts of fresh thyroid gland.

2. Iodothyrine in solution, injected intravenously, has little influence on the rate of cerebrospinal fluid secretion when given in small amounts (0.05 gm.). In amounts of 0.3 gm. and 0.5 gm. there is some inhibition of the rate of choroid plexus secretion, but not as marked as that produced by diiodotyrosine or saline extracts of fresh thyroid.

THE RESPONSE OF THE VASODILATOR MECHANISM TO WEAK, INTERMEDIATE, AND STRONG SENSORY STIMULATION

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In the study of vasomotor reflexes the desirability has long been recognized of supplementing by means of plethysmographic studies of individual organs the information gained from observations of blood-pressure changes. Recently Tschalussow[1] has described a method of employing the nasal cavity as a plethysmograph, and has found it to be of exceptional delicacy.

In certain studies from this laboratory the characteristic depressor effect of *weak* sensory stimulation has been emphasized,[2] and it has seemed to us worth while to supplement these studies with observations of the effects of weak sensory stimuli on the volume of the nasal cavity.

METHOD

Our experiments were performed on cats. In some cases urethane anesthesia was employed; in others rapid decerebration under ether was performed. The method of preparing the nasal cavity was the modification of Tschalussow's original one described by one of us.[3] Simultaneous blood-pressure records were made from a femoral artery. For sensory stimulation electrodes (Sherrington type) were placed centrally on branches of the brachial and sciatic trunks, and in some experiments on the vagus or saphenous. Induction shocks of known intensity (Z units[4]) were used for excitation. The rate varied between 5 and 15 per second.

[1] Tschalussow: Archiv für die gesammte Physiologie, cli, 1913, 524.

[2] Martin and Lacey: This Journal, xxxiii, 1914, 212; Martin and Stiles: ibid xxxiv, 1914, 106.

[3] Mendenhall: This Journal, xxxvi, 1914, 58.

[4] Martin: The measurement of induction shocks, New York, 1912, 73.

The nasal cavity is peculiarly adapted for an analysis of depressor reflexes, since it receives both vasodilator and vasoconstrictor fibres,[5] and can respond, therefore, to reflex influences acting either through the vasoconstrictor or the vasodilator central mechanism.

With our method of recording nasal volume-change, as in the experiments of Tschalussow (loc. cit), an up-stroke of the lever signifies vasodilation, and a down stroke vasoconstriction. Changes in nasal volume following stimulation of sensory nerves can be interpreted as signifying particular vasomotor effects only when recorded in connection with simultaneous records of general blood pressure, for only thus can passive changes be distinguished from active ones. Even when so accompanied, the distinction cannot always be made with certainty. To be sure, active nasal vasoconstriction occurring simultaneously with general vasoconstriction is readily distinguished, since here we have a down-stroke of the nasal recorder with upward movement of the blood-pressure tracing; likewise active nasal vasodilation in the presence of general vasodilation presents no difficulty since in this case there will be an upward stroke of the nasal recorder with downward movement of the blood-pressure tracing. The difficulty arises when we attempt to determine whether local active vasomotor changes may occur in the presence of general vasomotor changes of opposite sign. To determine whether the nasal vessels are actively dilating or are merely being gorged passively by rising general blood pressure requires the most careful analysis of the records.

For purposes of comparison we have found it desirable, as did Tschalussow,[6] to bring about known passive changes in nasal circulation. Means to this end are compression of the abdominal aorta, which brings about passive engorgement of the vessels of the head; or compression of the thorax, or peripheral stimulation of the cut vagus to cause the blood supply to the head to be lessened. In our experience the nasal cavity always responds immediately to these procedures. Tschalussow found (loc. cit., 52), that the nasal vessels react to passive engorgement by energetic local contraction. This we have seen in a few, but by no means in all, of our experiments. As an index of passive engorgement, therefore, we do not consider this after contraction wholly reliable.

Observations. In general the nasal volume changes brought about by stimulation of sensory nerves correspond with expectation. That is

[5] Langley: Philosophical Transactions, London, clxxxiii, 85; Dastre and Morat: Recherches sur le systeme nerveux vaso-moteur, Paris, 1884, 115 et seq.

[6] Tschalussow: Loc. cit., 527.

to say, weak stimuli, such as cause drop in general blood pressure, cause simultaneously nasal vasodilation, and strong stimuli, which cause a rise of pressure, produce nasal vasoconstriction. The nasal mucosa, therefore shares actively in the reactions by which both pressure-fall and pressure-rise come about.

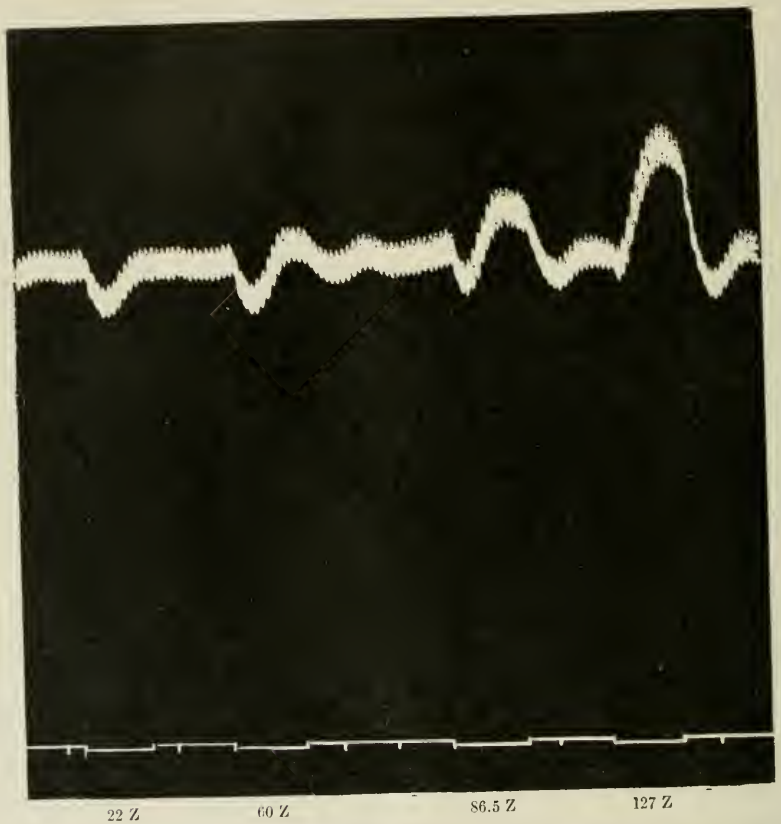


Fig. 1. The transition from a depressor to a pressor response with increasing strength of stimulation. The time signal is at the line of zero pressure. Time in 30 second intervals. Stimulation strengths in Z units. Peroneal nerve stimulated.

In the presence of a mechanism of this sort, in which weak stimulation produces one sort of response and strong stimulation of precisely the same nerve trunks a diametrically opposite response, a degree of interest naturally centres about the point of reversal, for here, at the

point where one sort of influence relinquishes control of the mechanism to another sort, we may hope to gain some insight into the nature and mode of action of the influences themselves. The great sensitiveness of the nasal plethysmograph lends itself to this possibility.

So far as we are aware the character of the blood-pressure curve under sensory stimulation near the reversal point has not hitherto been reported in detail, although it has been observed in this laboratory from the beginning of our quantitative study of vaso-motor reflexes, and has undoubtedly been observed incidentally by other investigators. A typical example of the transition from a depressor to a pressor reflex is presented in figure 1. As the strength of sensory stimulation is increased above that which brings about pure pressure-drop a tendency develops toward the replacement, during the period of stimulation, of the initial pressure-drop by a pressure-rise. This tendency becomes more and more marked, until, in most cases, with the application of sufficiently strong stimulation, the initial depressor phase is completely suppressed, leaving the rise of pressure as the only visible response. In the particular experiment illustrated in the figure the region of reversal occurred with somewhat weaker stimulations than we have ordinarily found adequate. The average of all our experiments places the "reversal thresholds" in the neighborhood of 200–250 Z units as compared with 60–90 in this experiment.

Fig. 2. Active nasal vasodilation persisting throughout a vasomotor reflex consisting of a depressor followed by a pressor phase. Peroneal nerve stimulated.

The responses of the nasal plethysmograph to sensory stimulation in the region of "reversal" are instructive. During the initial pressure drop there is active dilation of the nasal mucosa. As the depressor phase is succeeded by the pressor there is often a corresponding change to active constriction of the nasal vessels. This latter reaction is not, however, invariable. We have sometimes seen the active nasal dilation persist throughout the pressor phase, particularly when the latter was not very pronounced. Such a case is illustrated in figure 2. In this same experiment stronger stimulation brought about a typical pressor reaction with the usual active nasal constriction.

A further suggestive observation is that in a fair percentage of cases of ordinary pressor stimulation active constriction of the nasal mucosa does not begin at the instant o pressure-rise but several seconds thereafter. The latent period for the nasal mucosa, where a well marked one occurs, averages about 15 seconds. Obviously the discharge of the vasoconstrictor centre which brings about the pressure-rise does not, in these cases, involve immediately the nasal mucosa. A simple explanation would be that in this region, which is also under the control of the vasodilator mechanism, active vasoconstriction is held in abeyance for a time by a simultaneous vasodilator discharge.

From all these observations we may draw the inference that depressor influences and pressor influences can be aroused simultaneously by suitable stimulation of individual sensory nerve trunks, and that the resultant effect on the peripheral vasomotor mechanism depends on the balance that is established between the opposing influences. To put the point in other words, when strong sensory stimulation brings about a rise in blood pressure, it may be that depressor influences are not suppressed, but merely overpowered.

In any attempt to analyze the interaction of simultaneous pressor and depressor influences we have to bear in mind the potentially twofold nature of each. Pressor influences may act positively on the vasoconstrictor centre or negatively on the vasodilator, either separately, or, as Bayliss maintains,[7] on both together. Likewise depressor influences may operate either through lowering constrictor tone or heightening dilator tone or both. A method of separating these factors used by Bayliss (loc. cit.), and also by Fofanow and Tschalussow,[8] consists in cutting either the constrictor or the dilator efferent paths to the organ upon which plethysmographic studies are being made. By this means observed vasomotor changes can be assigned definitely to one or the other mechanism. We have applied this method in our experiments with the nasal plethysmograph in order to determine whether the depressor influences manifested during weak sensory stimulation involve activity of the vasodilator mechanism. We find that marked active nasal dilation is induced by weak stimulation when both cervical sympathetic nerves are severed. This procedure cuts the nasal mucosa off from its connection with the vasoconstrictor centre while leaving it in communication with the dilator mechanism through the

[7] Bayliss: Proceedings of the Royal Society of London, 1908, lxxx, B, 339.

[8] Fofanow and Tschalussow: Archiv für die gesammte Physiologie, cli, 1913, 543.

Vidian nerves.[9] The observed dilation is the result, therefore, of positive discharges through the vasodilator system.

By the same procedure we have attempted to show whether the depressor influences which we have pictured above as present but ineffective during strong sensory stimulation involve positive action of the vasodilator mechanism. We have repeated the observations of Fofanow and Tschalussow on the effects on the nasal mucosa of stimulating the vago-depressor trunk[10] and confirm their finding that strong stimulation of this trunk with both cervical sympathetics cut brings about active reflex vasodilation, indicating a positive action upon the vasodilator apparatus.

The attempt to demonstrate by this method activity of the vasodilator mechanism during ordinary pressor stimulation is rendered difficult by the circumstance that when the cervical sympathetic nerves are cut the nasal mucosa may respond passively to the general rise of blood pressure, giving, as already pointed out, a tracing that is difficult to distinguish from active vasodilation.

There are two features, however, in which active dilation may differ from passive. The first is in time relationships, the second in degree of response.

A passive nasal volume-change must necessarily *synchronize accurately* with the blood-pressure rise or fall which produces it. The *degree* of response, moreover, will show some relation to the amount of general pressure-change, unless, indeed, the local reaction described by Tschalussow occurs (loc. cit., 52), in which case the response is immediately identified thereby as a passive one. In an experiment in which both cervical sympathetics are cut, a nasal dilation during pressor stimulation, which definitely outlasts the general pressure rise; or a series of dilations of *equal* extent in connection with pressor responses of *different* amounts, indicate strongly, if they do not prove conclusively, that the dilator mechanism is active during strong stimulation of sensory nerves. Both these appearances we have encountered in the course of our work, and they have been sufficiently clean cut to satisfy us that they were not accidental. For example, in one experiment, strong sensory stimulation (900 Z units) applied for 30 seconds, caused a reflex rise of pressure which fatigued quickly; so that the pressure had returned to the original level 5 seconds before the period of stimulation was over. Well marked nasal dilation accompanied the response

[9] Tschalussow: Loc. cit., 539.

[10] Fofanow and Tschalussow: Loc. cit., 554.

and continued for four seconds after stimulation ceased, outlasting the period of heightened pressure a total of 9 seconds. In another experiment, one with an exceptionally low pressor threshold, sensory stimulation of 35 Z units caused a rise of pressure of 14 per cent, and stimulation on the same nerve of 127 Z units caused a rise of pressure of 29 per cent. The nasal dilation was slightly more pronounced in the first case than in the second. The mechanical limit of the nasal mucosa was not approached either time, for compression of the abdominal aorta, within 9 minutes of these observations, produced a passive nasal dilation nearly double the greater of them.

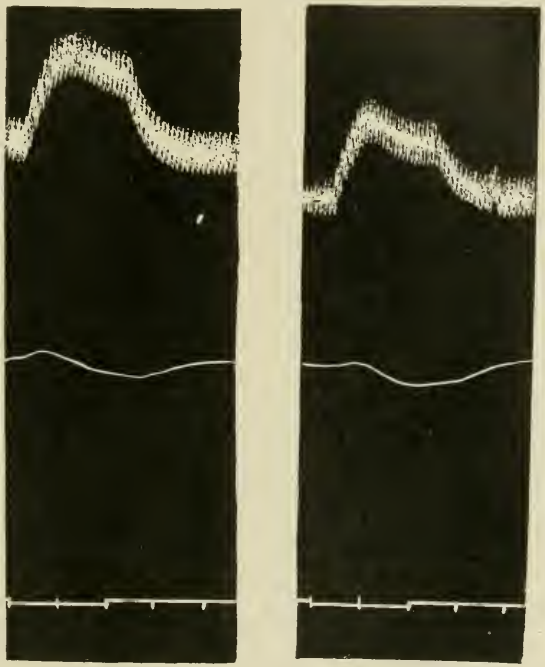
DISCUSSION

On the basis of the observations reported above, and those formerly reported from this laboratory we would suggest the following as a possible picture of the responses of the vasomotor mechanism to sensory stimulations of increasing strength. Weak sensory stimuli excite the vasodilator apparatus to activity. The result is a drop in general blood pressure, with active dilation in such organs as are provided with vasodilator nerves. Our data yield no positive information as to whether weak stimuli affect the vasoconstrictor centre; but if this centre is affected appreciably it is inhibited. As stronger and stronger sensory stimuli are used there begins to develop, concurrently with the active vasodilation, excitation of the vasoconstrictor centre. The latter is more potent than the concomitant dilation although its latency appears to be ordinarily somewhat longer, for the typical response to intermediate stimulation is an initial active dilation followed by, and overpowered by, a subsequent active constriction. With still stronger stimuli the active constriction develops with less delay, so that, save in exceptional cases, no indication is afforded of the vasodilator discharges which are occurring simultaneously with the predominant vasoconstrictor activity. When the sensory nerve stimulated is the vagodepressor trunk, the situation is the same, except that with this nerve strong stimulation inhibits the vasoconstrictor centre, reinforcing, instead of opposing, the concurrent dilator discharges.

This suggestion of concurrent excitation of both mechanisms is directly contrary to the view of Bayliss (loc. cit.), according to which vasoconstrictor excitation is accompanied by inhibition of vasodilator tone. Bayliss states that most of his attempts to demonstrate inhibition of dilator tone gave negative results.[11] He reports, however, two

[11] Bayliss: Loc. cit., 349.

experiments which he interprets as supporting his contention (loc. cit., 350). In the first of these, during a pressor reflex with rising general blood pressure, active vasoconstriction occurred in the ear of a rabbit both of whose cervical sympathetics had been cut. Since virtually all vasoconstrictor fibres were presumably severed Bayliss considers that this active vasoconstriction was brought about through inhibition of vasodilator tone. This interpretation involves the rather difficult mechanical conception that the arterioles are so forcibly held open through their dilator innervation that when this innervation subsides they will constrict forcibly against a rising blood pressure in the complete absence of constrictor excitation. The alternative possibility suggests itself that in this particular animal an aberrant vasoconstrictor innervation to the ears was present. As supporting this alternative we wish to cite an experiment of our own. On February 26, 1915, we were making observations on a cat under urethane anesthesia. After eliciting typical pressor responses from stimulation of the left peroneal and left radial nerves, with active nasal vasoconstrictions with each response, we cut both cervical sympathetics and repeated the sensory stimulations. Contrary to our experience in numerous other experiments, and to our great surprise, each pressor reflex was accompanied, precisely as before, by active nasal vasoconstriction instead of by the ex-



a *b*

Fig. 3. Active nasal vasoconstriction during pressor stimulation. *a*, cervical sympathetics intact; *b*, cervical sympathetics cut. Peroneal nerve stimulated.

pected dilation. We performed ten sensory stimulations, three on the left radial, four on the left peroneal, and three on the left popliteal with results as stated. Figure 3 gives two tracings from this experiment. In both of these the stimulation was applied to the peroneal; the strength of stimulus was 1300 Z. Stimulation *a* shows the response before the cervical sympathetics were cut; *b*, the response after they were cut. The interval between stimulations *a* and *b* was 12 minutes. The cervical sympathetics were cut 5 minutes after stimulation *a*. The animal was vagotomized, and we searched both carotid sheaths carefully for minute nerve strands that might represent portions of the cervical sympathetics separate from the main trunks. Comparison of the nasal tracings in *a* and *b* of the figure indicates strongly that if the first represents vasoconstrictor excitation the second does also. We consider aberrant vasoconstrictor innervation a much more probable explanation of this and of Bayliss' phenomenon than depression of dilator tone.

Bayliss' second experiment is one in which diminution of volume of the hind leg occurred in an animal, presumably with abdominal sympathetics cut, from stimulation of the median nerve, with simultaneous excitation of the depressor and the peripheral end of the cervical sympathetic. The conditions of stimulation in this experiment are such as to make the interpretation of the results difficult. An investigation now in progress in this laboratory on the effects of simultaneous stimulation of the depressor and other sensory nerves reveals a complexity of interaction requiring careful quantitative comparisons for analysis. The results of this investigation will be reported in due course. In this connection we wish to say only that we should hesitate to admit the possibility of inhibition of vasodilator tone during adequate stimulation of the depressor nerve, unless all other possibilities, such as aberrant vasoconstrictor innervation, or passive redistribution of blood, were more rigorously excluded than they appear to have been in this experiment.

SUMMARY

Depressor (weak) stimulation of sensory nerves produces active dilation of the vessels of the nasal mucosa.

Pressor (strong) sensory stimulation produces active constriction in the nasal mucosa.

Sensory stimuli of intermediate intensity produce typically an initial fall in general blood pressure followed by a rise. The nasal mucosa

shows active dilation during the initial depressor period. Sometimes the dilation persists through the second or pressor phase; at other times there is active nasal constriction during the pressor phase.

In many instances the active constriction of the nasal mucosa under pressor stimulation shows a longer latency than does the general blood pressure rise.

Both these observations suggest an active dilator influence which is able for a time to oppose successfully the constrictor influence occurring simultaneously.

When both cervical sympathetic nerves are cut weak sensory stimulation produces active dilation of the vessels of the nasal mucosa, showing that the vasodilator mechanism is excited by weak stimuli.

We confirm the observation of Fofanow and Tschalussow that with strong stimulation of the vago-depressor trunk, when the cervical sympathetics are cut, the vasodilator mechanism is excited.

Observations are reported which indicate that the nasal dilation seen when strong (pressor) stimulation is applied to ordinary sensory nerves, with both cervical sympathetics cut, is, in part, at least, the result of excitation of the vasodilator mechanism.

On the basis of these experiments the suggestion is made that the vasodilator apparatus is ordinarily excited whenever sensory nerve trunks are adequately stimulated. Strong stimuli, by exciting concurrently the vasoconstrictor mechanism, may overpower the dilation in regions where both innervations obtain.

Although this suggestion is based altogether upon the results of artificial stimulation of sensory nerve trunks and contains no implication that *receptor* stimulation may not be selective as regards the elicitation of vasodilator or vasoconstrictor responses independently, it may not be amiss to point out that an important feature of the physiological significance of the high blood pressure induced by vasoconstriction is in the diversion of much of the blood of the body into those organs which appear to have a vasodilator innervation but no constrictor, namely, the skeletal muscles. This distribution of the blood would be favored by the mechanism here postulated.

SPINAL ANAESTHESIA IN THE CAT

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The dangers and discomforts of ether anaesthesia have long been recognized. Inevitable nausea, possible pneumonia, difficult respiration, renal injuries, make ether anaesthesia impossible in certain cases, dangerous in others, and distressing in all. To meet these difficulties, spinal anaesthesia was devised for use in fields where general anaesthesia is superfluous, such as operations on the perineum. As a rule beneficent, spinal anaesthesia is nevertheless by exception open to a grave and sudden danger. In the course of surgical procedures otherwise completely successful, the vasomotor apparatus may suddenly give way. The fall in blood pressure is immediate, sometimes profound, always disquieting. Nor can the surgeon predict in what patient it may appear.

The points of interest in this phenomenon are as follows: (1) The extent and the time relations of the fall in blood pressure and the effect of this fall on the efficiency of the central nervous system. (2) The region paralyzed. (3) The structures affected, whether the vasomotor center, the roots of the spinal nerves, the afferent or the efferent paths in the body of the spinal cord. (4) The extent to which the drug may pass along the cord from the point of injection, as modified by the per cent of the drug in the solution used, the bulk of this solution, the force of gravity, and the possible fixation of the drug by the tissues which it bathes. (5) The duration of the phenomena. (6) The influence of adrenalin. (7) Remedial measures, directed to raising the fallen blood pressure.

Obviously, these factors cannot be studied with complete satisfaction in man, in whom the condition of experimentation cannot be varied at will. We present, therefore, a systematic investigation of spinal anaesthesia in animals.[1]

[1] The only experimental study of blood pressure in spinal anaesthesia so far as we are aware, is that of Gray and Parsons (Quarterly Journal of Medicine, 1911, v, p. 339), who concluded that the slight fall of blood pressure they obtained in all cases was due to relaxation of the muscles of the abdomen and the lower limbs and that the greater fall obtained in some cases was due to paralysis of the intercostal muscles and the consequent diminution in the pumping power of the chest.

METHOD

Fifty cats were used. In a number of these, two or more intraspinal injections were made, so that, in all, 72 experiments were done. In 18 cases, ether alone was used. In all the animals, the preliminary operations were done under ether anaesthesia. In 32 cases, in which muscular reactions would have been a vital source of error, ether was followed by curare. Enough dilute curare solution to paralyze the skeletal muscles was slowly injected through the femoral vein. The carotid blood pressure was recorded by a membrane manometer. Graduation scales for this manometer are shown in figures 1, 2 and 3. The condition of the vasomotor system and of the sensory afferent tracts was determined by measuring the changes in blood pressure on stimulation of the central end of the brachial and sciatic nerves and on stimulation of the dorsal columns of the cord. The induction currents employed were just perceptible to the tongue. In order to make sure that the drug entered the subdural space, the injection was made under the guidance of the eye. Laminectomy, therefore, was always done at the level of the injection. To determine the spread of the drug by direct stimulation of the cord, laminectomy was often done at other levels as well. A 2 cc. all-glass Luer syringe with 24 gauge needle was used. In the cat, the space between the cord and the dura is so shallow that, except in the lower lumbar region, the needle cannot be inserted perpendicularly to the long axis of the cord without impaling the cord itself. The direction of the needle, whether pointing cephalad or caudad, was found to be a factor of some influence on determining the level to which the drug diffused.

In most of the experiments undertaken to ascertain the effect of gravity, the foot of the board was raised.

In 35 injections, tablets "C" containing 0.05 g. novocaine and 0.000083 g. adrenalin were used; in 18 injections, tablets "D" containing 0.2 g. novocaine and 0.06 g. sodium chloride; in 17 injections, a fluid preparation put up in ampoules, each of which contained 1.3 cc. of 5 per cent tropacocaine and 0.00017 g. suprarenin chloride; and in 2 injections, a fluid preparation in ampoules containing 1 cc. of 5 per cent tropacocaine in 0.6 per cent sodium chloride solution. Taking as a test the paralysis of the tissue directly bathed by the drug when injected, the novocaine with adrenalin gave 28 per cent unsatisfactory results, ranging from partial to complete failure; novocaine and salt, 38 per cent; tropacocaine and adrenalin, 23 per cent; only two injections were made with tropacocaine and salt and both

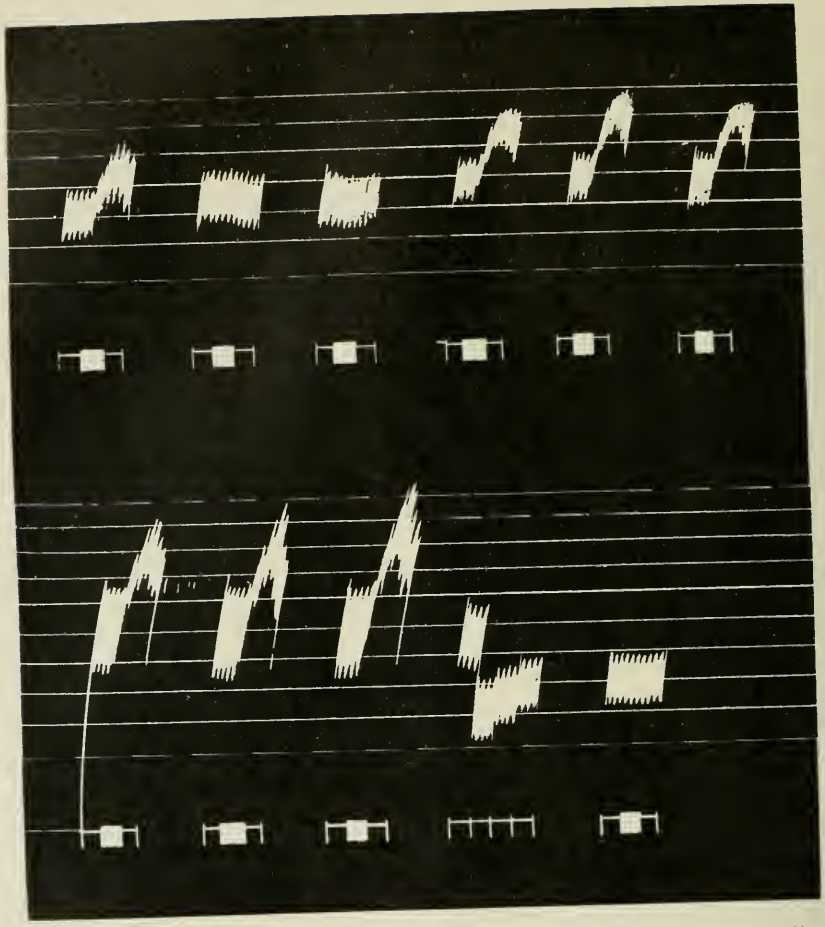

Fig. 1. The original size. Injection of 0.01 g. novocaine and adrenalin in dilute solution (1 cc.) at Lumbar VII causes paralysis of dorsal columns extending to Dorsal XI, and perhaps above. Brachial rise reduced from 65 to 33 per cent.

Lower curve—left to right		*Upper curve—left to right*	
1. Sciatic stimulation	12.10 p.m.	1. Brachial stimulation	12.35 p.m.
2. Brachial stimulation	12.14	2. Lumbar VII stimulation	12.37
3. Lumbar VII stimulation	12.15	3. Dorsal XI stimulation	12.39
4. Record of blood pressure	12.17	4. Sciatic stimulation	3.25
	(12.22 Injection of drug)	5. Lumbar VII stimulation	3.27
	12.25	6. Dorsal XI Stimulation	3.29
	12.28		
	12.31		
5. Sciatic stimulation	12.32		

Scale: 50, 70, 90, 110, 130, 150, mm. Hg.

Experiment 45, curarized cat. February 26, 1915.

were successful. The percentages of failure were considerably higher than in the clinical use of the same drugs, a difference probably due to the conditions obtaining in experimental work on so small an animal, and to the use of inadequate doses in certain experiments.

Following is a typical protocol.

Experiment February 26, 1915. A lightly etherized cat was tracheotomized and cannulas placed in the left carotid artery and the left femoral vein. The left brachial and sciatic nerves were tied and cut distal to the ligature. Laminectomy was done at Lumbar VII and Dorsal XI. The carotid cannula was connected with a membrane manometer.

11.50 a.m. 1.2 cc. 0.5 per cent curare solution in 15 cc. normal saline solution injected into femoral vein.[2] Artificial respiration.

12.10 p.m. Sciatic nerve stimulated with induction currents. The blood pressure rose from 105 mm. to 170 mm. Hg. (see fig. 1). Stimulation of brachial nerve and of dorsal columns at Lumbar VII gave slightly greater increase.

12.17. Blood pressure recorded.

12.22. 1.0 cc. of 1.0 per cent novocaine and adrenalin C (made with distilled water) was injected very slowly into the dural sac at Lumbar VII. The needle was inserted perpendicularly to the long axis of the cord.

12.25. Blood pressure has fallen from 100 mm. to 55 mm.

12.28. Blood pressure 65 mm.

12.31. Blood pressure 71 mm.

12.32. Stimulation of sciatic nerve. The reflex change in blood pressure has disappeared.

12.35. Brachial stimulation causes a rise from 75 to 100 mm. (33 per cent) instead of the rise from 100 mm. to 165 mm. (65 per cent) shown before novocaine.

12.37. No reflex rise on stimulating cord at Lumbar VII

12.39. No reflex rise on stimulating cord at Dorsal XI.

3.25. Stimulation of sciatic, brachial, Lumbar VII, and Dorsal XI cause normal reflex increase in blood pressure.

In all the experiments, especial care was taken to avoid errors from ether, curare, and artificial respiration.[3]

CHANGES IN BLOOD PRESSURE

Extent of fall. In Table 1 are recorded 20 experiments in which novocaine or tropacocaine was injected in the lumbar region in strength sufficient to block all afferent impulses set up by stimulation of the sciatic nerve. In two cases, the blood pressure fell more than 40 per cent. A fall of 40 per cent is, however, not necessarily alarming.

[2] This small dose of curare is excreted after artificial respiration has continued some time and the curare must then be renewed.

[3] For precautions, see W. T. Porter: this Journal, 1910, xxvii, pp. 281, 282.

TABLE 1

NO.	SITE OF INJECTION	BLOOD PRESSURE	ABSOLUTE FALL	PERCENTILE FALL
	Lumbar	*from to*		
12	I	138–105	33	24
20	III	100– 90	10	10
25	VI	150–100	50	33
26	VII	130–100	30	23
28	VII	140–130	10	7
29	VII	100	0	0
30	VII	130–140		
31	VII	80– 70	10	12
32	VII	120–100	20	8
33	VII	170–140	30	18
34	VII	130–120	10	8
39	VII	90	0	0
40	VII	100– 80	20	20
42	VII	120– 95	20	17
44	VII	160–100	60	38
45	VII	100– 55	45	45
47	VII	100– 70	30	30
48	VII	150– 80	70	47
49	VI	120– 90	30	25
50	VII	190–170	20	11
	Dorsal			
2	XIII	120– 80	40	33
3	IX	130– 80	50	38
4	II	115– 30	85	74
5	IV	120– 75	45	38
6	IV	100– 40	60	60
7	IV	100– 40	60	60
8	IV	120– 75	45	38
9	IV	100– 90	30	30
13	I	110– 60	50	45
21	II	120– 40	80	67
22	X	130– 60	70	53
26	XII	115– 80	35	30
27	XII	80– 50	30	38
29	XI	100– 90	10	10
32	XII	120– 75	45	38
34	X	120– 30	90	75
35	IX	110– 80	30	27
37	XI	80– 70	10	13
43	VI	110– 45	60	55
	Cervical			
14	III	125– 70	55	44
15	III	100– 80	20	20
16	III	90– 60	30	33
17	III	95– 40	55	58
18	III	80– 60	20	25
19	IV —	120– 80	40	33
23	III	80– 55	25	31
36	III	110–160		
36	III	100– 60	40	40
38	III	110–100	10	9
38	III	120–110	10	8
38	III	100– 90	10	10
46	II	130– 70	60	46

The criterion is not the absolute or percentile fall of blood pressure per se, but whether there remains sufficient blood pressure to carry on, for a time at least, the work of nerve cells in the brain and cord. In one of the two cases just cited, the pressure fell from 150 mm. to 80 mm. Hg, in the other it fell from 100 mm. to 55 mm. The danger line may probably be placed at 60 mm. In only one instance out of twenty, therefore, was there a serious fall in consequence of a lumbar injection.

In the dorsal region, 19 injections were made, in 9 of which the blood pressure fell to 60 or less.

In the cervical region, there were 13 injections and in 5 the blood pressure fell to 60 or below.

In the cat the residual blood pressure, after the extirpation of the spinal cord[4] is from 28 to 31 mm. In our present experiments, the lowest blood pressure after lumbar injection was 55 (one instance); after dorsal injection, the pressure fell in two cats to 30 mm. and in three cats to 40 mm.; after cervical injection, the pressure fell once to 40 mm. In several instances, therefore, the vasomotor apparatus was absolutely paralyzed and the function lost as completely as if the spinal cord had been extirpated. These instances were, in each case, the result of injection in the dorsal or cervical regions.

Duration of low blood pressure. The injury to nerve cells caused by low blood pressure and the consequent impaired nutrition, depends on two variables; namely, the extent to which the pressure falls and the duration of the low pressure. Recovery from the slight falls in blood pressure usually took place rapidly. After the more severe falls, partial but sufficient recovery took place in from 30 to 90 minutes. The duration of low blood pressure appeared to depend more upon the amount of drug injected than upon the site of injection.

Our experiments give evidence that in the majority of instances the vasomotor system was not seriously impaired by the pressures noted in Table I. After 70 to 180 minutes the reflex change in blood pressure on stimulation of the sciatic and brachial nerves returned almost, if not quite, to normal. Thus in Experiment 43, sciatic stimulation caused the blood pressure to rise from 90 to 145 mm., 61 per cent. After injection at Dorsal VI, the pressure fell from 110 to 45 mm. Seventy minutes later, the blood pressure was 70 mm. and on sciatic stimulation it rose to 110 mm., 57 per cent.

[4] Porter and Storey: this Journal, 1907, xviii, p. 196.

THE REGION PARALYZED

It is obvious that drugs like novocaine, which are given to interrupt the afferent conducting paths in the spinal cord, may also interrupt the efferent paths. The object in view is to suspend sensations of pain without at the same time suspending some function essential to the well-being of the patient. Such injuries must depend on the importance of the several regions paralyzed. Reflection upon the anatomy of the spinal cord will show that the vasomotor and the respiratory functions are especially to be considered.

The vasomotor function. The reader is reminded that the master cells controlling the tonus of the arteries and thus the weight of the blood pressure are situated in the bulb. Their axis cylinder processes descend the cord in the antero-lateral tracts, bend into the gray matter, and end there in contact with the spinal vasomotor cells. The axis cylinder processes of the spinal vasomotor cells leave the cord in the anterior roots of spinal nerves from the first Dorsal to about the first Sacral. The vascular areas served by these fibers are most of them without importance in the present investigation. Thus in the cat, the sciatic nerve, bearing vasomotor fibers for the hind limb, may be severed without causing any considerable fall in blood pressure. In fact, only the splanchnic nerves, given off from the upper dorsal region, innervate a vascular area large enough to be a dangerous factor in spinal anaesthesia. This area, comprising the abdominal viscera, is, however, so extensive as to make splanchnic paralysis very serious, in that the dilatation of the arteries controlled by the splanchnic vasomotor fibers may cause so much blood to enter the corresponding veins that not enough is left in the bulb and cord to support properly respiration and other vital functions. The rabbit, for example, may be bled to death into its own portal system, by section of the splanchnic nerves. In studying the regions affected by spinal anaesthesia, special attention should, therefore, be paid to that containing the splanchnic fibers. Spinal anaesthesia can be successful, only when the afferent paths are paralyzed without at the same time paralyzing enough splanchnic cells or splanchnic root fibers to lower the blood pressure to a degree that may threaten the continued activity of the centers situated in the cervical cord and the bulb.

The importance of the splanchnic area becomes clear when attention is directed to the average percentile fall in blood pressure after injections in the several regions of the cord (Table 1). The averages are: lumbar, 19 per cent; dorsal, 43 per cent; and cervical 27 per cent.

The regional averages just presented, show that the fall in blood pressure, following dorsal injection, is due to paralysis in the splanchnic region and not to paralysis of the bulbar vasomotor center. For, if the fall were due to interference with the bulbar center or with the vasomotor fibers connecting it with the spinal vasomotor cells, the use of novocaine in the cervical region, nearer the bulbar center, should

TABLE 2

Maximum fall in blood pressure in relation to dosage, region injected, and direction of injection

Fall of 30 Per Cent or Less						Fall of More Than 30 Per Cent					
EXPERIMENT NO.	BULK	PER CENT	LEVEL	NEEDLE TOWARDS	PER CENT FALL	EXPERIMENT NO.	BULK	PER CENT	LEVEL	NEEDLE TOWARDS	PER CENT FALL
	cc.						*cc.*				
17	0.2	4	C. III	Head	5	46	0.2	5	C. II	Head	46
18	0.3	4	C. III	Head	18	16	0.4	2	C. III	Tail	33
						4	0.4	2.5	D. II	Tail	73
9	0.1	1	D. IV	Head	10	8	0.1	1	D. IV	Tail	37
35	0.4	2.5	D. IX	Spine	27	5	0.3	2	D. IV	Tail	37
12	0.5	2	L. I	Tail	24	7	0.1	2	D. IV	Tail	60
11	0.4	2	L. II	Tail	24	6	0.2	2	D. IV	Tail	60
10	0.2	2	L. III	Head	25	43	0.2	5	D. VI	Spine	54
49	0.2	5	L. VI	Spine	25	3	0.5	2	D. IX	Head	38
47	0.5	2	L. VII	Head injected forcibly.	30	2	0.5	1	D. XIII	Head	33
42	0.2	5	L. VII	Spine	16	25	0.2	2.5	L. VI		33
50	0.2	10	L. VII	Spine	10	44	1.0	1.5	L. VII	Spine	37
						45	1.0	1.0	L. VII	Spine	45
						48	0.8	2.5	L. VII	Spine	47

In all the above experiments a mixture of novocaine and adrenalin C. was used.
"Spine" means that the needle was pointed at right angles to the long axis of the cord.

SUMMARY

	Injection away from splanchnic area	*Injection in splanchnic area*
	11 Experiments	*14 Experiments*
Average dose	= 0.0093 g.	= 0.0082 g.
Average bulk	= 0.3 cc.	= 0.42 cc.
Average per cent	= 3.6 per cent	= 2.3 per cent
Average blood pressure fall	= 19.5 per cent	= 45.2 per cent

cause as great a fall as its use in the dorsal region—at any rate, the fall should not be less.

The conclusion that the vasomotor paralyses of spinal anaesthesia are to be sought in the splanchnic area rather than in the bulbar vasomotor center is further supported by the observations on the fall of blood pressure as affected by the direction in which the injection is made.

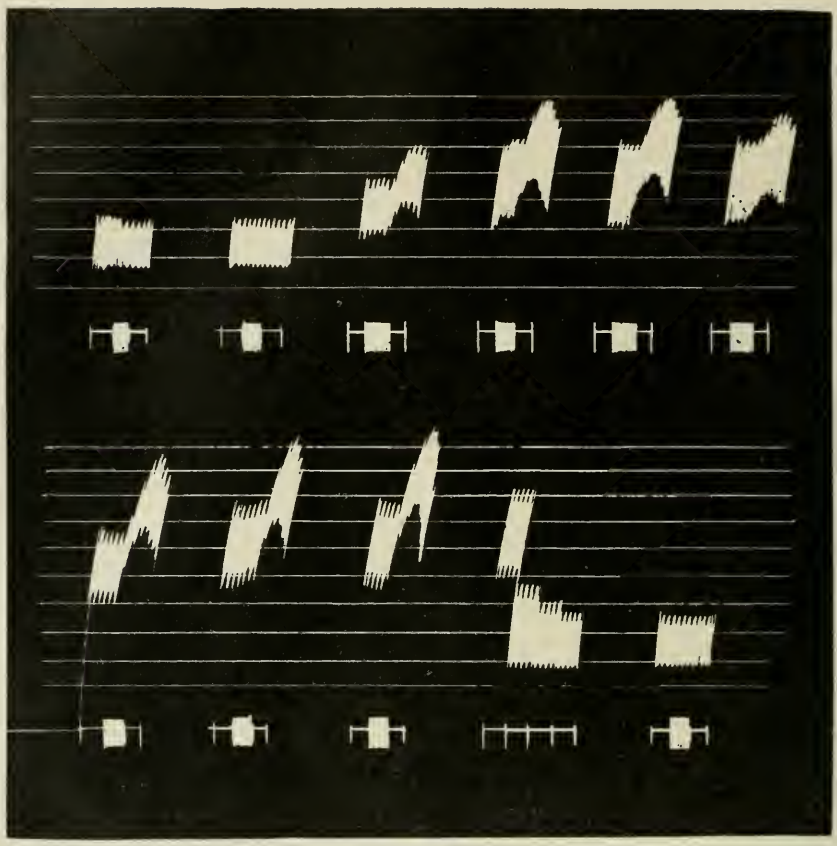

Fig. 2. The original size. Injection of 0.01 g. novocaine and adrenalin (0.2 cc.) at Dorsal VI causes fall in blood pressure from 110 mm. to 45 mm. in three minutes with abolition of vasomotor reflex from sciatic and brachial.

Lower curve—left to right		*Upper curve—left to right*	
1. Sciatic stimulation	8.55 p.m.	1. Brachial stimulation	9.15 p.m.
2. Brachial stimulation	8.58	2. Dorsal VI stimulation	9.17
3. Dorsal VI stimulation	9.01	3. Brachial stimulation	9.42
4. Record of blood pressure	9.02	4. Brachial stimulation	10.12
	(9.03 Injection of drug)	5. Sciatic stimulation	10.14
	9.06	6. Dorsal VI stimulation	10.15
	9.09		
	9.12		
5. Sciatic stimulation	9.13		

Scale: 30, 50, 70, 90, 110 ,130, 150, mm. Hg.

Experiment 43, curarized cat. February 19, 1915.

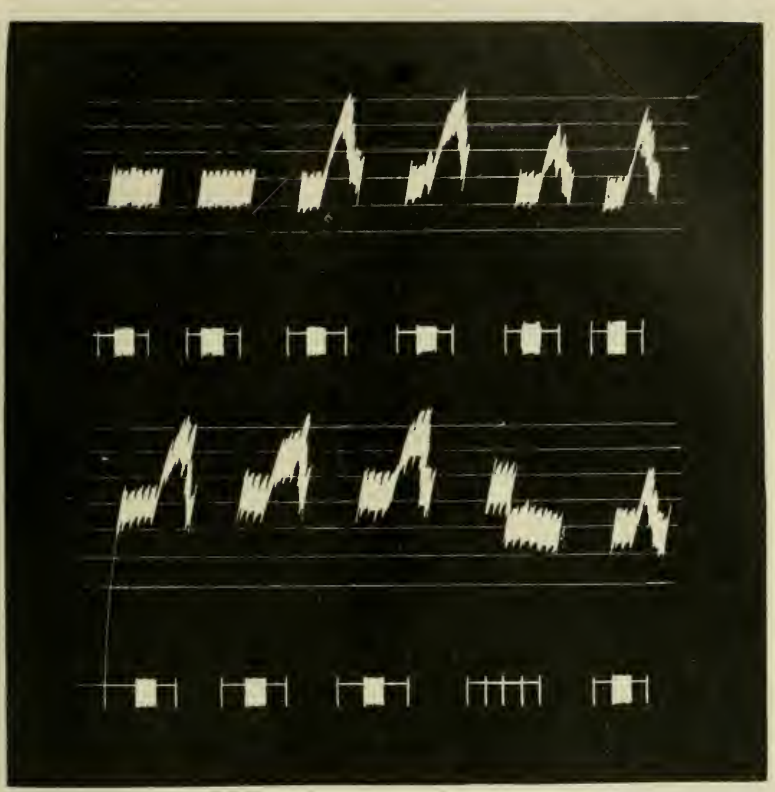

Fig. 3. The original size. Injection of 0.01 g. novocaine and adrenalin (0.2 cc.) at Lumbar VII causes fall of blood pressure from 120 mm. to 95 mm. in three minutes. Paralysis of sciatic incomplete eleven minutes after injection. Elevation of foot of board at angle of 30°, eighteen minutes after injection, is followed by complete paralysis of sciatic nerve.

Lower curve—left to right		*Upper curve—left to right*	
1. Sciatic stimulation	9.11 p.m.	1. Sciatic stimulation	9.48 p.m.
2. Brachial stimulation	9.13	2. Lumbar VII stimulation	9.49
3. Lumbar VII stimulation	9.15	3. Lumbar I stimulation	9.51
4. Record of blood pressure	9.17	4. Brachial stimulation	9.54
	(9.22 Injection of drug)	5. Sciatic Stimulation	10.22
	9.25		
	9.28		
	9.31		
5. Sciatic stimulation	9.33		

Scale: 70, 90, 110, 130, 150, 170, 190 mm. Hg.

Experiment 42, curarized cat. February 16, 1915.

Table 2 deals with the fall of blood pressure in relation to dosage, region injected, and the direction of the injection. On the left side of this table are placed the cases in which the fall of blood pressure was 30 per cent or less; on the right side are those in which the fall was more than 30 per cent of the initial pressure. In every case in which the fall was 30 per cent or less (except Experiment 35), the injection was so made that the drug was driven away from the area included between Dorsal I and IX (figs. 2 and 3). Contrast Experiment 8 with Experiment 9; in both, 0.1 cc. of 1 per cent novocaine and adrenalin was injected at Dorsal IV. In Experiment 8, in which the drug was driven towards the tail, the fall was 37 per cent; in Experiment 9, in which the solution was driven towards the head, the fall was 10 per cent. Again, in Experiment 16, 0.4 cc. of 2 per cent solution was injected at Cervical III caudad; the fall was 33 per cent; in Experiment 4, 0.4 cc. of 2.5 per cent solution, injected caudad from Dorsal II, much nearer the fall-producing area, was followed by a fall of 73 per cent. The conclusion again appears justified that with moderate but adequate doses, the fall in blood pressure in spinal anaesthesia is caused by paralyses in the splanchnic area.

The clinical use of spinal anaesthesia is limited to the injection of the drug in the lumbar region. As the drug diffuses towards the head, the first part of the vasomotor mechanism affected by it will be the roots in the thoracic area. It seems justifiable to assume that in clinical, as well as in experimental spinal anaesthesia, the fall of blood pressure is caused by paralysis of the splanchnic area.

Paralysis of respiration. Out of a total of 18 experiments in which no curare was used, the injection was made in the cervical or upper thoracic region ten times. In four of these ten injections, the drug was driven towards the tail from a point below the phrenic nerve and there was no paralysis of respiration. In the other six, the drug was injected towards the fifth cervical level; in four of these cases, respiration was paralyzed. In the other two cases, the dosage was very small (0.1 cc. of 1 per cent, 0.1 of 2 per cent novocaine and adrenalin.)

In closing this discussion of the regions affected by spinal anaesthesia, it is important to answer the very practical question, How often will surgical anaesthesia of the lumbar and sacral region be complicated by a serious fall in blood pressure or by an interruption of the breathing? In our experiments, there was but one case out of twenty lumbar injections in which the fall in blood pressure (to 55 mm.) might have

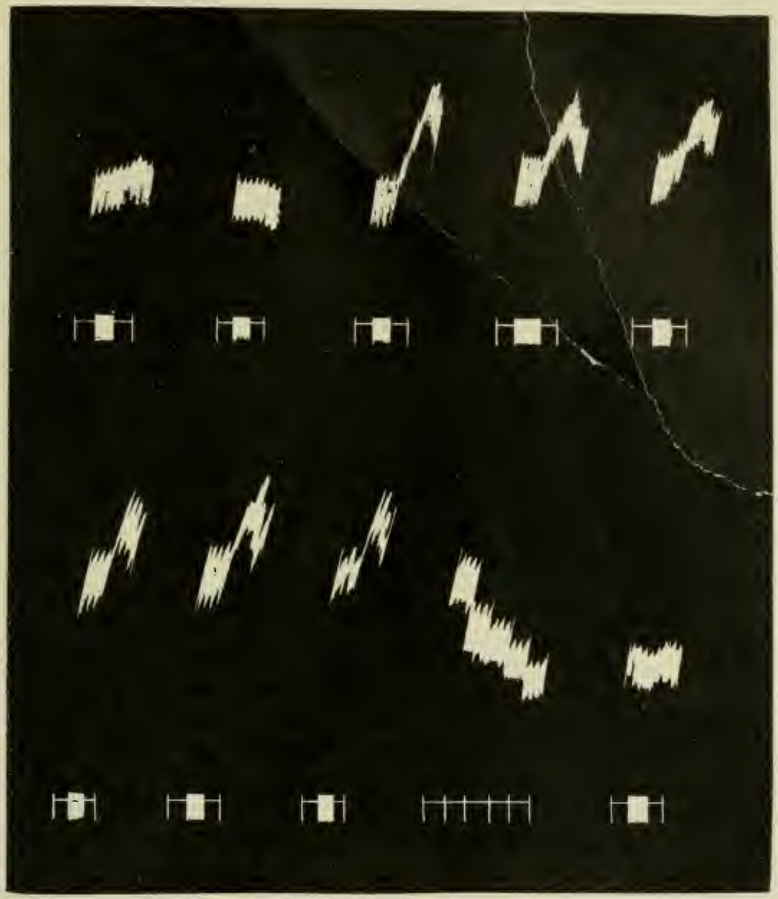

Fig. 4. The original size. Injection of 0.01 g. novocaine and adrenalin (0.2 c.c.) at Cervical II cephalad causes fall of blood pressure which is gradual rather than abrupt, due probably to slower action of drug on cord itself than on thoracic roots. Dorsal columns blocked, but vasomotor mechanism below D I is unaffected.

Lower curve—left to right		*Upper curve—left to right*	
1. Sciatic stimulation	9.40 p.m.	1. Brachial stimulation	10.05 p.m.
2. Brachial stimulation	9.43	2. Cervical II stimulation	10.07
3. Cervical II stimulation	9.46	3. Dorsal II lateral surface stimulation	10.12
4. Record of blood pressure	9.48	4. Sciatic stimulation	11.40
	(9.49 Injection of drug)	5. Brachial stimulation	11.43
	9.52		
	9.55		
	9.58		
	10.01		
5. Sciatic stimulation	10.03		

Experiment 46, curarized cat. March 4, 1915.

been serious, and in the eight injections in which no curare was used there was no paralysis of respiration.

THE STRUCTURES AFFECTED

The usual site of paralysis being in the splanchnic area, we should now enquire whether the drug affects the anterior nerve roots or the paths bringing vasoconstrictor impulses from the bulb.[5]

Since paralyses of respiration are so infrequent in spinal anaesthesia, we have not attempted to differentiate paralysis of the phrenic root fibers from that of the bulbo-phrenic respiratory path.

It may at once be stated that a strength of the drug sufficient to paralyze the afferent sensory paths (so that stimulation of the central end of the sciatic nerve produces no reflex) will also paralyze the efferent vasomotor fibers (fig. 4). This is illustrated by Experiment 23, in which 0.5 cc. of 2.5 per cent tropacocaine and adrenalin were applied to all surfaces of the cord at Cervical II. The dura at that level was laid open. The blood pressure fell from 80 to 55 mm.; stimulation of the sciatic and brachial nerves and the anterior surfaces of the cord at Cervical III produced no response. Stimulation of the anterior surface of the cord at Dorsal II, however, was followed by an excellent rise in blood pressure, thus proving the integrity of the vasomotor mechanism below the paralyzed portion.

It is possible, on the other hand, to secure paralysis of the nerve roots without disturbing the conductivity of the vasomotor paths in the substance of the cord, as in Experiment 15. In this cat, 0.2 cc. of 4 per cent novocaine and salt solution "D" was injected at Cervical III. The stimulation of the sciatic caused the blood pressure to rise from 80 mm. to 140 mm., but brachial stimulation caused no rise. The brachial roots in this experiment were paralyzed but the afferent paths conveying sciatic impulses remained unaffected.

There is some evidence to show that different functions may be affected differently. For example, Experiments 2, 3, and 8 showed that the motor paths are paralyzed more easily than the sensory paths

Experiment 2. 0.5 cc. of 1 per cent novocaine and adrenalin was injected at Dorsal XIII. Stimulation of left sciatic nerve followed by rise in blood pres-

[5] We have at present no satisfactory method of isolating effects limited to the splanchnic cells, if indeed the cells are ever paralyzed independently of the nerve paths.

sure throughout experiment, whereas right hind leg was completely paralyzed for 55 minutes.[6]

Experiment 8 shows that the vasomotor reflex may persist although spontaneous motion of the extremities is lost.

Experiment 8. In an etherized cat, 0.1 cc. of 1 per cent novocaine and adrenalin was injected at Dorsal IV at 12.04 p.m. At 12.31, 12.36 and 12.44, left sciatic stimulation was followed by rise in blood pressure from 105 to 120, 100 to 115, and 110 to 120. The right hind leg remained paralyzed for 65 minutes.

THE DIFFUSION OF THE DRUG ALONG THE CORD

In studying the diffusion of the drug along the spinal cord,[7] it seemed well to fix a reasonable interval between the moment of injection and the testing of the resultant paralysis. This period was set at fifteen minutes, in which time the drug seemed to have exerted its maximal effect. Care was taken not to manipulate the spine after the injection, lest the fluid injected should be pumped or driven to a more distant level.

Per cent of drug. In the following experiments, the same quantity of solution was injected, but the solution contained different amounts of anaesthetic (a constant mixture of novocaine and adrenalin "C"). Paralysis of the dorsal column to direct stimulation was the test employed to fix the limits to which the drug had spread.

Experiment 25. 0.225 cc. of 2.5 per cent (0.0056 g.) at Lumbar VII diffused 6 vertebrae.

Experiment 42. 0.2 cc. of 5 per cent (0.01 g.) at Lumbar VII did not diffuse 5 vertebrae.

Experiment 49. 0.2 cc. of 5 per cent (0.01 g.) at Lumbar VI did not diffuse 2 vertebrae.

Experiment 50. 0.2 cc. of 10 per cent (0.02 g.) at Lumbar VII did not diffuse 4 vertebrae.

The average diffusion here was less than four vertebrae.

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[6] S. Baglioni (Centralblatt für Physiologie, 1910, xxiii, pp. 869–873), has shown that after the subdural injection of stovain, sensations disappear in this order: pain, cold, heat, pressure; they return in reverse order. This seems to show a varying degree of resistance to the effect of drugs.

[7] Some writers in this field emphasize movements of the spinal fluid due to the effects of respiration upon the emptying and filling of the cerebro-spinal venous system. That this possible factor in the diffusion of the drug is done away with when the dorsal sac is opened to atmospheric pressure, an operation found essential in our experiments, we are not prepared to deny. Other, and more important, factors affecting distribution within the dural sac can be studied by our method and indeed the problem is simplified by the removal of confusing influences.

Varying bulk of fluid. In the following experiments the amount of fluid injected was varied, while the percentage of the drug (novocaine and adrenalin "C" remained the same. The paralysis of the dorsal columns was again the test of diffusion.

Experiment 25. 0.225 cc. of 2.5 per cent (0.0056 g.) at Lumbar VII diffused 6 vertebrae.

Experiment 24. 0.25 cc. of 2.5 per cent (0.0062 g.) at Lumbar VI diffused 6 vertebrae.

Experiment 34. 0.4 cc. of 2.5 per cent (0.01 g.) at Dorsal II diffused 9 vertebrae.

Experiment 35. 0.4 cc. of 2.5 per cent (0.01 g.) at Dorsal IX diffused 11 vertebrae.

Experiment 48. 0.8 cc. of 2.5 per cent (0.02 g.) at Lumbar VII diffused 8 vertebrae.

The average diffusion was eight vertebrae.

Additional information is afforded by certain experiments in which the drug was injected at approximately the same levels (Lumbar VI or VII) but in which dilute and concentrated solutions are examined with regard to their effect upon blood pressure. The greater the fall, the further the drug progressed toward the splanchnic area.

Dilute solutions

Experiment 48. 0.8 cc. of 2.5 per cent (0.02 g.) caused blood pressure to fall 46 per cent.

Experiment 44. 1.0 cc. of 1.5 per cent (0.015 g.) caused blood pressure to fall 37 per cent.

Experiment 45. 1.0 cc. of 1.0 per cent (0.01 g.) caused blood pressure to fall 45 per cent.

Experiment 47. 0.5 cc. of 2.0 per cent (0.01 g.) caused blood pressure to fall 39.5 per cent.

The average fall was 41.9 per cent. The average dose was 0.014 g.

Concentrated solutions

Experiment 50. 0.2 cc. of 10 per cent caused blood pressure to fall 11 per cent.

Experiment 41. 0.3 cc. of 5 per cent caused blood pressure to fall 16 per cent.

Experiment 49. 0.2 cc. of 5 per cent caused blood pressure to fall 25 per cent.

Experiment 42. 0.2 cc. of 5 per cent caused blood pressure to fall 17 per cent.

Experiment 40. 0.2 cc. of 5 per cent caused blood pressure to fall 20 per cent.

The average fall was 17.8 per cent. The average dose was 0.013 g.

The analysis of the observations on diffusion does not show any very definite laws, probably because the number of experiments is

necessarily limited. On the whole, the bulk seemed a factor of greater importance than the strength of the solution. Dilute solutions seemed to spread further than concentrated solutions. But in some cases, a dose of small bulk and containing a small amount of the drug produced a more widespread effect than a dose larger both in bulk and in percentage of drug injected in a manner as nearly similar as possible.

Effect of gravity. We are aware of the possibility of error in attempting to determine the effect of gravity upon the diffusion of a drug injected into a dural sac which is exposed at the highest point to atmospheric pressure, whereas normally the cord is protected by its bony envelope. To avoid this source of confusion as far as possible, we tilted the animal and allowed the blood pressure and spinal fluid to become settled after the change of position, before injecting the drug. After the injection, the board was left tilted for fifteen minutes, then returned to level and the dorsal columns were stimulated to determine the extent of the diffusion.

The following experiments compare three animals in the horizontal position with four in which the head was tilted down at a varying angle.

Horizontal

Experiment 42. 0.2 cc. 5 per cent novocaine and adrenalin C at Lumbar VII, did not diffuse 5 vertebrae.

Experiment 49. 0.2 cc. 5 per cent novocaine and adrenalin C at Lumbar VI, did not diffuse 2 vertebrae.

Experiment 29. 0.2 cc. 5 per cent novocaine D at Lumbar VII, diffused 3 vertebrae.

Tilted, head down

Experiment 31. 0.2 cc. 5 per cent novocaine D at Lumbar VII, diffused 6 vertebrae.

Experiment 32. 0.2 cc. 5 per cent tropacocaine and adrenalin at Lumbar VII, diffused 7 vertebrae.

Experiment 39. 0.2 cc. 5 per cent novocaine D in 5 per cent glucose at Lumbar VII, diffused 8 vertebrae.

Experiment 40. 0.2 cc. 5 per cent novocaine C in 5 per cent glucose at Lumbar VII, diffused 8 vertebrae.

It appears that tilting the animal board at an angle of 40, head down, increases the diffusion of novocaine and salt solution, and that the diffusion is increased to a slight degree when the drug is carried in a 5 per cent glucose solution.

Fixation of the drug. The complete fixation of the drug in some loose chemical combination with the tissues of the cord would be greatly

to the advantage of the surgeon. If such a bond existed, the action of the anaesthetic would soon be localized. If the paralysis had extended far enough to affect seriously the blood pressure, the patient could then be tilted head down, thus keeping by the force of gravity a supply of blood in the brain. If, however, it can be demonstrated that the drug is not entirely fixed, it would follow that tilting the patient might cause the unfixed remainder of the anaesthetic to flow towards the head, invading more of the splanchnic region, and even reaching the phrenic cells, and finally the spinal bulb.

We present three observations upon fixation:

Experiment 34. One cc. of 1.0 per cent tropacocaine and salt solution was injected at Lumbar VII. Twenty minutes later, stimulation of Lumbar I produced a fair rise in blood pressure. Five minutes after that, the dura at Lumbar III was opened. Evidently the manipulation drove the drug upwards, for after that Lumbar I no longer reacted.

Experiment 38. 0.4 cc. of 5.0 per cent novocaine and salt solution D was injected at Cervical III. Blood pressure fell from 100 to 90 mm., but returned to 100 mm. in 13 minutes. Sixteen minutes after the injection, the dura was opened at Dorsal X and as the spinal fluid flowed down the cord the blood pressure fell from 100 to 80.

Experiment 40. 0.2 cc. of 5.0 per cent novocaine and adrenalin C was injected at Lumbar VII. Paralysis of the sciatic did not occur. Eighteen minutes after the injection, the board was tilted and immediately afterwards complete paralysis of the sciatic was found to have taken place.

From these three experiments we may conclude that after 25, 16, and 18 minutes respectively, enough drug free from fixation was present to paralyze other nerve fibers.

THE DURATION OF THE PHENOMENA

The duration of paralysis of the vasomotor reflexes was studied in relation to the absolute amount of drug injected, and also in relation to the percentage of the drug in solution. In many cases, a low dosage or one of weak percentage (1 to 2 per cent) secured as long a paralysis as did stronger or larger doses. The minimum dose could not be determined with any finality, for in one experiment 0.1 cc. of 1 per cent solution would be fairly effective, while in another a much larger dose would not give the desired effect.

In Experiments 26, 27, 29, and 30, the spinal fluid was drained off 15 minutes after the injection of the solution, but this did not shorten the duration of the paralysis.

In Experiments 5, 6, 8, 20, 21, 22, 24, and as the effects of the drug began to wear off, the stimulation of the sciatic nerve was followed by a fall of blood pressure instead of a rise. In cases in which this phenomenon occurred, the normal reflex returned before the blood pressure rose to its original level.

THE INFLUENCE OF ADRENALIN

In order to learn whether adrenalin was a factor in the phenomena following spinal anaesthesia, we twice injected adrenalin chloride alone.

Experiment 37. 0.5 cc. of 1–10,000 adrenalin chloride was injected cephalad from Dorsal XI. No change in blood pressure followed, but sciatic reflex was temporarily diminished, perhaps because the solution was cool.

Experiment 38. 0.5 cc. 1–10,000 adrenalin chloride, warmed, was injected caudad from Cervical III. The blood pressure fell from 120 to 110 in five minutes. The reflexes were not affected.

A comparison of the action of novocaine and adrenalin C with that of novocaine and salt solution D is given in Table 3. The injection was caudad in Experiment 15 and cephalad in all the others.

TABLE 3

Comparison of action of novocaine + salt ("D") and novocaine + adrenalin ("C")

EXPERIMENT	DOSE	LEVEL	TIME OF ONSET ON BLOOD PRESSURE	PER CENT OF BLOOD PRESSURE FALL	DURATION OF PARALYSIS		PARALYSIS DORSAL COLUMNS	PARALYSIS OF ROOTS
					of blood pressure	of reflexes		
				per cent				
15-D	0.2 cc. of 4% (caudad)	C. IIJ	3	20	25	26	No	Yes
17-C	0.2 cc. of 4% (cephalad)	C. III	3	5	5	0	No	No
29-D	0.2 cc. of 5%	L. VII	0	0	0	25		Yes
42-C	0.2 cc. of 5%	L. VII	3	16	60+	60+	Yes	Yes
30-D	0.2 cc. of 5%	L. VII	Rise		0	27	Yes	Yes
49-C	0.2 cc. of 5%	L. VI	9	25	23	28+	Yes	Yes
36-D	0.2 cc. of 5%	C. III	Rise			35	Yes	?
46-C	0.2 cc. of 5%	C. II	12	46	110+	110	Yes	?
38-D	0.4 cc. of 2.5%	C. III	0.5	9	4	20	No	Yes
16-C	0.4 cc. of 2%	C. III	3	33	16+	0	No	Yes
39-D	0.2 cc. of 5% glucose	L. VII	0	0	0	105+	Yes	Yes
40-C	0.2 cc. of 5% glucose	L. VII	3	20	60	60	Yes	Yes

Table 3, showing six pairs of experiments, exhibits a markedly greater fall of blood pressure in the cases in which adrenalin was used. This occurs in five of the six pairs. In the remaining pair, the fall of blood pressure with novocaine and salt is not great (20 per cent). We do

not attempt to explain this fact. It may be that the pressure of salt in solution D is a factor.

It is also noteworthy that although in three experiments the novocaine D was more effective as regards duration of paralysis of the reflexes, the average duration of the paralysis after use of D was 40 minutes, whereas, of the four experiments in which C produced paralysis at all, the average duration was 64 minutes. The longest action of D was secured when the solution was made up in 5 per cent glucose. C failed twice to produce paralysis; D never failed. On the whole, the honors seem to be divided fairly evenly.

MEASURES TO RAISE THE FALLEN BLOOD PRESSURE

In five experiments an effort was made to raise the lowered blood pressure by the intravenous injection of salt solution, adrenalin chloride or pituitrin. This was done both when the blood pressure was lowered by section of the cord, and by spinal anaesthesia. To be of value, such experiments should be done only after section of the cord as otherwise the natural return of blood pressure as the spinal drug wears off will influence the results.

Saline solution, in the two experiments in which it was injected intravenously, did not materially affect the blood pressure.

The effect of pituitrin and adrenalin were tried with the cord cut across at Cervical III. Blood pressure stood at 50 in Experiment 36, at 55 in Experiment 38. In Experiment 36, 0.5 cc. pituitrin in 5 cc. H_2O was given. The blood pressure rose in one minute from 50 to 100, and four minutes later had fallen again to 60.

In Experiment 38, 0.5 cc., 1-10,000 adrenalin chloride in 5 cc. NaCl was given. The blood pressure rose from 55 to 160 at once, but in four minutes after the injection was back at 50.

CONCLUSIONS

1. In our experiments on spinal anaesthesia, there was in twenty animals but one case in which a moderate but adequate injection in the lumbar region caused a fall in blood pressure that might have been serious (to 55 mm.); and in the eight cases in which no curare was used there was no paralysis of respiration after lumbar injection.

2. Even after marked falls in blood pressure partial but sufficient recovery took place in from 30 to 90 minutes. The duration of low blood pressure appeared to depend more upon the amount of drug injected than upon the site of the injection.

3. The fall in blood pressure seen after lumbar and dorsal injection is due to paralysis in the splanchnic region. In our numerous observations, it was not due to paralysis of the bulbar vasomotor center.

4. A strength of the drug sufficient to paralyze the afferent sensory paths in the cord (so that stimulation of the central end of the sciatic nerve produces no reflex) will also paralyze the efferent vasomotor fibers.

5. The nerve roots may in some cases be paralyzed without disturbing the conductivity of the vasomotor paths in the substance of the cord.

6. There is some evidence that different functions may be affected differently; thus in three experiments the motor paths were paralyzed more easily than the sensory paths.

7. Regarding the diffusion of the drug, the bulk seemed on the whole a factor of greater importance than the strength of the solution. Dilute solutions usually but not always spread further than concentrated solutions.

8. Gravity is a factor of some importance; tilting the animal at an angle of 40°, head downward, increased the diffusion of the drug.

9. Fixation of the drug is only partial. In three experiments, after 25, 16, and 18 minutes respectively, enough remained free to paralyze other nerve fibers.

10. In seven experiments, as the effect of the drug began to wear off, the stimulation of the sciatic nerve caused a fall of blood pressure instead of the usual rise. In these cases, the normal reflex rise returned before the blood pressure attained its original level.

11. A greater fall of blood pressure occurred in the cases in which adrenalin was used in connection with tropacocaine or novocaine.

12. Measures taken to raise the fallen blood pressure were of little value. It was easy to restore the blood pressure to normal but the normal level could be maintained but a few minutes.

THE CONDUCTION WITHIN THE SPINAL CORD OF THE AFFERENT IMPULSES PRODUCING PAIN AND THE VASOMOTOR REFLEXES

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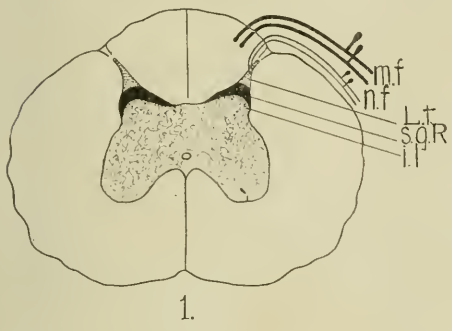
One of the problems which has been puzzling investigators for many years is that of the varieties of cutaneous sensation. How are impressions of pain, touch, heat and cold differentiated, and how are the underlying afferent impulses propagated along peripheral nerves and the spinal cord. So far as the peripheral nerves are concerned the problem has remained very obscure, and yet in the last decade advances have been made in our knowledge of the physiology and histology of afferent nerve fibers which promise to throw some light on the question.

On the physiological side Head (1) has shown that cutaneous sensations fall into two groups. In general it may be said that pain and temperature correspond to his "protopathic" group and light touch to his "epicritic." He has shown that there must be two kinds of cutaneous afferent nerve fibers corresponding to his two types of sensation. Fibers of one kind mediate protopathic sensation; fibers of the other sort mediate epicritic sensation. These two kinds of fibers differ as to anatomical distribution and rate of regeneration.

On the histological side Ranson (2) has shown that there are two kinds of afferent nerve fibers, the medullated and non-medullated. Although the non-medullated afferent nerve fibers are very numerous, in some nerves more than twice as numerous as the medullated, they remained unknown, until in 1911 a differential axon stain was developed and applied to the peripheral nerves. A remarkable parallel exists between Head's account of the protopathic fibers and the facts which have already been ascertained in regard to the non-medullated fibers. Space does not permit us to make a detailed comparison. We will restrict ourselves to a comparison of their course in the spinal cord. Head's protopathic fibers after entering the cord turn at once into the

gray matter. The epicritic fibers ascend for longer or shorter distances in the posterior funiculus before they enter the gray matter. This difference in the arrangement of the protopathic and epicritic fibers in the cord corresponds exactly to the difference in the course of the medullated and non-medullated dorsal root fibers. While most of the medullated fibers enter the posterior funiculus, all the non-medullated fibers turn at once lateral-ward into the tract of Lissauer in the apex of the posterior gray column (fig. 1.) Their course up or down the cord in Lissauer's tract is very short, the length of one or two cord segments. There is good reason to believe that they terminate in the substantia gelatinosa Rolandi (fig. 1), and that this is the nucleus of reception of these non-medullated afferent fibers.



1.

Fig. 1. Diagrammatic section of the spinal cord of the cat at the level of the first lumbar segment. *m.f.*, medullated dorsal root fibers; *n.f.*, non-medullated dorsal root fibers; *L.t.*, Lissauer's tract, *s.g.R.*, substantia gelatinosa Rolandi; *i.l.*, intermediate layer.

On the basis of many such points of similarity between the protopathic and the non-medullated afferent fibers one of us formulated the theory (Ranson 1914) that the non-medullated afferent fibers conveyed pain and temperature sensation. It was suggested that the tract of Lissauer, the substantia gelatinosa Rolandi, and the bundle of non-medullated fibers in the ventrally lying intermediate layer (fig. 1) constituted a mechanism for the reception and conduction of pain and temperature sensations. It was further suggested that this assumption "does not exclude the possibility that we are dealing here with a center for vasomotor and pilomotor control, as suggested by Sano. In fact these autonomic functions are of necessity closely correlated with the afferent impulses which find their conscious expression in the form of sensations of pain, heat and cold. It is thus possible that the apparatus in question has a double function, serving as a central autonomic apparatus and for the reception and conduction of pain and temperature sensations."

It has been pretty well established that pain is transmitted up the cord in the anterior part of the lateral funiculus (Van Gehuchten (3),

Petrén (4), Piltz (5), Bertholet (6), May (7) and Rothman (8)). Ziehen (9) however stated that pain is conducted upward in the apex of the posterior horn; and we were inclined, because of our studies on the tract of Lissauer, to believe that he was correct. At least it seemed probable that the apex of the posterior horn was an important part of the mechanism for the reception, and conduction of the "nociceptive" (Sherrington (10)) afferent impulses which are represented in consciousness by the sensation of pain.

With such possibilities in mind we determined to make a careful study of the conduction within the cord of the nociceptive afferent impulses. Such a study divides itself into two parts, dealing first, with the conduction of these impulses to the cortical centers for conscious pain, and second, with the conduction of these impulses to the centers for the autonomic reflexes, notably the vasomotor center. It will be clear that the paths in the cord for the conduction of these impulses to the cerebral cortex on the one hand and to the autonomic centers on the other need not necessarily be the same. The study of paths in the spinal cord for the nociceptive afferent impulses as determined by the ordinary tests for pain and as determined by the vasomotor reflexes forms two mutually supplementary lines of investigation which have been carried out by us on the same series of animals. The results of both lines of investigation can, therefore, be directly compared.

TECHNIQUE

Cats were used throughout this series of experiments. Various spinal cord lesions were produced, and after recovery each cat was subjected to two kinds of tests to determine the effect of the lesion, first, on the conduction of pain; and second, on the character of the vasomotor reflexes. To avoid possible variations according to age only full grown cats were used.

The operations were made at the level of the first lumbar segment, which lies under the spine of the second lumbar vertebra. A very high grade of asepsis was maintained. In addition to the usual procedures to secure asepsis, three special precautions were taken. All skin was excluded from the operative field by sterilized oiled muslin placed under the laparotomy-sheet and slit to correspond to the skin incision. The cut edges of the skin and the margins of the slit in the muslin were held together by skin clips in such a way that no skin could be seen through the opening in the laparotomy-sheet. The operator's

fingers were kept out of the wound, and gauze, needle and sutures were handled only with instruments. In closing the wound a layer of superficial fascia was drawn over the line of closure in the deep fascia and sutured to the deep fascia half an inch beyond this line. This layer of superficial fascia becomes adherent to the deep fascia in a few hours and forms a perfect protection for the deeper part of the wound. In a few cases the cutaneous incision was torn open by the cats, but in none of the twenty-five did infection develop beneath this layer of imbricated superficial fascia.

The dura was exposed and opened in the usual way. Dorsal and lateral hemisections and sections of the posterior funiculis were made with a small sharp knife. The apex of the posterior horn was destroyed on both sides of the cord in six cats by dissecting with a specially designed probe between the dorsal and lateral funiculi. All lesions were made in the first lumbar segment, except in four of the earlier experiments, in which by mistake the lesion was in the second and one in which it was in the third lumbar segment.

Attempts to produce ventral hemisections were unsuccessful. Each of the three trials resulted in serious injury to the lumbrosacral cord, probably due to a disturbance of its blood supply. From all the other operations the animals recovered promptly and moved about normally after periods varying from two days to two weeks, except in the case of cat A3 in which there was permanently some disturbance of motion and some atrophy of the hind limbs. The cats gained in weight and were in excellent condition when their vasomotor reflexes were tested.

The pain sense was tested in the unanaesthetised animals by pricking with a needle and by pinching the skin. These crude methods of inducing pain were regularly supplemented by the use of the cutaneous needle electrodes, used by Martin, Porter and Nice (11) in determining the "sensory threshold for faradic stimulation in man." Faradic stimulation of uniform strength through these electrodes gives much more uniform and dependable pain reactions than can be obtained by pinching or pricking the skin. The cord lesion was at the level of the first or second lumbar segments and hence above the level of origin of all the nerves going to the hind limb. All tests of the hind limbs were made over the area of distribution of the sciatic nerve which takes origin below the level of the fifth lumbar segment. A sharp cry and generalized struggling movements were taken as criteria of pain.

After complete recovery of the animal from the lesion and after the conduction of pain in the cord had been tested the vasomotor reflexes were studied. Under ether anaesthesia a tracheotomy was performed and the ether bottle attached. Connections were made as rapidly as possible for carotid blood pressure and respiratory tracings. Both sciatic nerves were exposed, ligated and cut distally to the ligature. The central end was laid bare for some distance above the ligature and could be handled by the attached thread. On the left side three brachial nerves, the median, ulnar and internal cutaneous were exposed, ligated together, and cut distally to the ligature and thereafter treated as a single nerve. Care was taken not to stretch the nerves at any time. When not being stimulated, they were kept covered with other tissues to prevent drying.

To eliminate passive dilatation of blood vessels in the areas supplied by the divided nerves the limbs were constricted with heavy cord placed proximal to the elbow and knee joints. Fluctuations in blood pressure from pressure on the abdomen by flexion of the limbs during stimulation of a nerve were prevented by securely tying the legs to the animal board.

The stage of ether anaesthesia is of the greatest importance as the vasomotor reflexes are affected by the slightest overdose, (Porter (12)). This seems to be especially true of the depressor reflex which is difficult to obtain under deep anaesthesia. The animal was kept relaxed, with regular respiration, with pupils half contracted, and with a brisk corneal reflex. Any tendency toward cyanosis was prevented because asphyxia, being a powerful vaso-constrictor stimulus, would obscure the results of sciatic stimulation (Sollman and Pilcher (13)).

Faradic stimulation of the central ends of the cut nerves was used to elicit the reflexes. Standard platinum electrodes were applied at least half an inch from the cut ends of the nerves, held suspended by the threads with which they had been ligated. The electrodes were moved slowly along the nerve during stimulation. The source of the current was a Stoelting inductorium No. 7090 through the primary of which passed a constant half ampere current. The primary current was obtained by shunting the instrument circuit across part of the resistance of a two ampere one hundred ten volt direct current "individual unit" system (von Hess (14)) attached to an ordinary lamp socket. By actual observation at repeated intervals the current through the primary coil was found to be very constant. The rate of interruption of the primary current was made fairly slow, about 25 per

second, in order to avoid possible overlapping of the currents induced in the secondary coil (Erlanger 1914). A knife switch in the primary circuit insures uniform contact. It is believed that with this system the strength of the induced current for each position of the secondary coil does not vary greatly from day to day. In this way stimulation of the same strength can be applied to several nerves in the same cat and to the nerves of different cats and the results compared.

Curare was used in some of the experiments. It was injected intravenously very slowly, until the stimulation of the peripheral end of a cut nerve gave no response.

After the reflex vasomotor and respiratory responses had been recorded the animal was killed, the cord exposed and the level of the lesion accurately determined. A stretch of cord about seven millimeters long containing the lesion was removed and prepared by the pyridine-silver method (Ranson (2)) and cut into serial sections. The serial sections were then studied to determine the extent of the lesion.

LITERATURE ON THE VASOMOTOR REFLEXES

There is a vast number of articles on the conduction of pain in the spinal cord. Good reviews of this literature have been given by Bertholet (6), May (7) and Karplus and Kreidl (15).

Considerable work has been done on the vasomotor reflexes, but relatively little is known concerning the anatomical location of the reflex arcs involved. It is generally stated that there exists in the brain stem a center for the regulation of blood pressure. It is supposed that this is primarily a vasoconstrictor center and is associated with the pressor reflexes. Little or nothing is known concerning the location of a center or centers for the depressor reflexes. In addition to the "principal" vasomotor center in the bulb "secondary" segmental centers are located in the thoracic and upper lumbar portions of the spinal cord, i.e., in those segments of the cord with which the white rami are associated.

Concerning the paths in the cord along which afferent impulses ascend to the vasomotor centers little is known. Dittmar (16) obtained the usual pressor effects from sciatic stimulation after section of the posterior columns and gray matter of the spinal cord. Sherrington (17) obtained results which led him to conclude that the afferent path to the vasomotor center lay in the anterior part of the lateral funiculus and states that Miescher had previously obtained similar

results. Bikeles (18) also reports some experiments which led him to the same conclusion. None of these papers give evidence of a thoroughgoing investigation of the question and the results are open to numerous objections such as the testing of the vasomotor reflexes before the animal has recovered from the effects of the operation, the location of the lesion too high (9th Th. Seg.) and the failure to distinguish between pressor and depressor reflexes.

Aside from these experiments little has been done to determine the effects of cord lesions on the vasomotor reflexes. Stursberg (19) showed that complete destruction of the cord at the level of the 7th thoracic segment involved the fibers which produced a coördination of the vasoconstrictors of the arm and leg.

The observations of Sherrington (10), Pike (20) and Porter and Muhlberg ('21) on "spinal shock" have an important bearing on our work. After transection of the spinal cord at the 8th cervical segment or during complete cerebral anaemia the blood pressure falls but soon returns to normal. The vasomotor reflexes are at first abolished but return in a short time. According to Sherrington the pressor reflex becomes very good after a few weeks and must now be purely spinal.

It has recently been shown that stimulation of the central end of the sciatic produces depressor or pressor reflexes according to the strength of the stimulus. (Porter (12), Sollman and Pilcher (22), Martin and Lacey (22).) Weak stimulation gives depressor reflexes, strong stimulation gives pressor reflexes. Martin and Lacey argue that the depressor reaction from sciatic stimulation is the more normal, since the pressor reactions are produced only by excessive stimulation.

VASOMOTOR REACTIONS IN NORMAL CATS

In order to determine the character of the normal vasomotor reactions we obtained tracings from fourteen normal adult cats. In all of these, excepting two, stimulation of the sciatic or brachial nerves with strong currents (s.c. 4 to 6 = secondary coil at 4 to 6) gave an increase in blood pressure, Table I. This pressor response varied considerably in extent. The cat giving the least response showed a rise in blood pressure of 11 mm. The greatest reaction obtained from these normal cats was a rise of 48 mm.

Stimuli in the depressor range (s.c. 13 to 18) were given to nine of these normal cats. They all showed a drop in blood pressure varying in extent from 4 to 22 mm. Hg. With medium strengths of current the results varied greatly, some cats showing a rise others a fall in blood pressure. These results corroborate those of Porter (12), Sollman and Pilcher (22) and Martin and Lacey (23) that stimulation of the central ends of afferent spinal nerves gives depressor reflexes with weak and pressor reflexes with strong stimulation. In the theoretical discussion of our results we will attempt to give an explanation of

TABLE I.

Normal cats.

CAT	INITIAL BLOOD PRESSURE	CHANGES* IN BLOOD PRESSURE IN MM. HG. FOR INDICATED POSITIONS OF SECONDARY COIL							
		17-18	15-16	13-14	11-12	9-10	7-8	5-6	4-5
N. 4	142								+13
N. 5	129								+ 9
N. 6	139								+23
N. 7	140								+11
N. 8	121				+18	+14			+48
Ex. 1	122	− 8	−11	− 5	−22	−14	−12	−11	
Ex. 2	144	−15			+45			+38	
Ex. 3	130	− 9	−12	−18	−18	−14	−11	+24	+10
Ex. 4	154	−22			−28	−26		+24	+24
Ex. 5	124	− 6	− 4		−10			+14	+ 8
Ex. 6	171	− 8			−18	−22		−16	
Ex. 7	144	−22				−20		+24	+16
Ex. 8	153	− 3	− 6					+20	
Ex. 9	178	−10	−20					+38	

*A rise in blood pressure is indicated by +, a fall by −.

this change in direction of the reflexes with increasing strengths of stimulation.

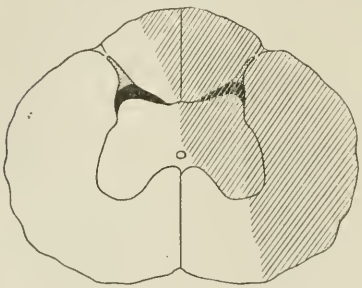
In our series of normal cats the sciatic nerve was used almost exclusively. Occasionally, however, tests were made on the brachial nerves—median, ulnar and internal cutaneous being stimulated alone or together. So far as our results show there was no difference in the reflexes obtained from sciatic and brachial nerves. This agrees with the observations of Porter and Richardson (24), who found by a long series of experiments with stimuli of given intensity, applied to the sciatic and brachial nerves in cats and other animals, that the result did not depend in any

way on which afferent spinal nerve was stimulated. These experiments were all with strong stimuli in the pressor range. Weak stimulation also gives a depressor response which is independent of the individual nerve stimulated, (Martin and Lacey (23)).

LATERAL HEMISECTIONS

The vasomotor reflexes were tested on five cats which had suffered a right lateral hemisection of the spinal cord 17 to 63 days before the tracings were taken. All five had recovered perfectly and had full use of both hind legs.

Post mortem examination showed the lesion located in the first lumbar segment in three of these cats and in the second lumbar segment in two. In one (cat O 7) the lesion when studied microscopically proved to be a perfect hemisection. In all the others part or all of the right anterior funiculus escaped injury (fig. 2). In two the lesion extended slightly beyond the mid line posteriorly into the left posterior funiculus (fig. 2). Since the conduction of pain and the reflex changes in blood pressure were the same in cat O 7 in which the hemisection was a perfect one as in the others in which the lesion was incomplete ventrally we conclude that these variations in the lesion have not affected the results.



2.

Fig. 2. Diagram of the second lumbar segment of cat O 8. The shaded area shows the part involved in the right lateral hemisection.

As far as could be determined by pricking the skin and by the use of cutaneous needle electrodes pain was conducted up the cord equally well from all four legs. We had hoped that by the use of the cutaneous electrodes we could locate accurately the pain threshold and determine if there were any difference on the two sides of the body below the lesion. We found that the threshold on the normal limb varied considerably and these normal variations were greater than any difference between the two hind limbs or between the front and the hind limbs in cats with lateral hemisection of the cord. We do not deny that in the cat some difference may exist in the conduction of pain from the two sides after lateral hemisection but such difference as may exist is too small to be readily recognized. In the same way we found it impossible

TABLE II

Depressor reflexes after lateral hemisection

CAT	POSITION OF SECONDARY COIL	NERVE	DROP IN BLOOD PRESSURE IN MM. HG. Individual tests	AVERAGE
0–6 I. B. P. 166	16	Brachial	22 18	20
		Right sciatic	24 22	23
		Left sciatic	5 5 8 5	6
	14	Brachial	20 19 31 27	24
		Right sciatic	21 19 25 26	23
		Left sciatic	5 5 14 11	9
0–7 I. B. P. 166	16	Brachial	21 19	20
		Right sciatic	21 20	21
		Left sciatic	−4 −5 0 5	−1
	14	Brachial	20 18 31 28	24
		Right sciatic	21 18 27 26	23
		Left sciatic	3 3 13 9	7
0–8 I. B. P. 142	12	Brachial	20 19 13	17
		Right sciatic	19 18 7	15
		Left sciatic	9 −3 9 9	6
	8	Brachial	16	16
		Right sciatic	23	23
		Left sciatic	5	5
A–4 I. B. P. 166	18	Brachial	15 15 16	15
		Right sciatic	18 13 11 0	11
		Left sciatic	7 8 9	8
	16	Brachial	14 23 22	20
		Right sciatic	20 10 12 8	13
		Left sciatic	16 13 9 5	11
A–5 I. B. P. 176	17	Brachial	12	12
		Right sciatic	15	15
		Left sciatic	5	5

to demonstrate any loss in sensibility to pain in the hind limbs as compared to the front limbs.

It is clear that the conduction of pain in the cat's cord takes place bilaterally. This agrees with the results of other observers on animals (Bertholet (6) and May (7)). But it is usually stated that, while

TABLE III

Pressor reflexes after lateral hemisection

CAT	POSITION OF SECONDARY COIL	NERVE	RISE IN BLOOD PRESSURE IN MM. HG. Individual tests	AVERAGE
0–6 I. B. P. 123	5	Brachial	5 0 0	2
		Right sciatic	0 0	0
		Left sciatic	6 6 14	9
0–7 I. B. P. 166	5	Brachial	29× 32×	31×
		Right sciatic	0 0 0	0
		Left sciatic	9× 10× 16×	12×
0–8 I. B. P. 142	4	Brachial	12	12
		Right sciatic	0	0
		Left sciatic	8	8
	2	Brachial	15× 0 16	10
		Right sciatic	0	0
		Left sciatic	10	10
A–4 I. B. P. 158	6	Brachial	28× 25×	27×
		Right sciatic	8×	8×
		Left sciatic	11	11
	4	Brachial	26× 32×	29×
		Right sciatic	5 0 −2	1
		Left sciatic	4 0 0	1
A–5 I. B. P. 176	5	Brachial	22× 31×	27×
		Right sciatic	0	0
		Left sciatic	9 9× 9	9

× indicates that the rise was followed by a considerable fall in blood pressure.

conduction of pain is bilateral in animals, it is more contralateral than homolateral. It is usually said that after a hemisection in animals there is partial analgesia of both hind limbs and that this is most noticeable on the side opposite the lesion. Karplus and Kreidl (15) have recently shown that after section of both halves of the cord in the cat at different levels, pain is still felt in the hind limbs. This would indicate that in the cat short spinal paths take an especially important part in pain conduction. We were unable to demonstrate any hypalgesia in the hind limbs of our cats with laterally hemisected cords. Despite these negative results as to the conduction of pain, these cats

showed characteristic departures from normal in their vasomotor reactions.

As will be seen by a study of Table II the depressor reactions obtained from the brachial and right sciatic nerves were normal and approximately equal. They were of the same average extent as the drops obtained in normal cats and showed no greater variation than these. In striking contrast is the reaction obtained from the left sciatic which was as a rule not more than a third as great as that obtained from the brachial and right sciatic nerves (fig. 3.). Each nerve was stimulated several times and the results of the individual tests were very consistent as

3.

Fig. 3. Blood pressure tracings from cat O 8, with right lateral hemisection of the cord. Base line raised 57 mm. *a*, drop of 18 mm. Hg. on stimulation of the left brachial nerves with weak faradic current *s.c. 14.; b*, drop of 19 mm. Hg. on stimulation of the right sciatic nerve with weak faradic current *s.c. 14, c*, negligible drop on stimulating the left sciatic nerve with weak faradic current *s.c. 14.*

will be seen by the table. Each of the five cats gave the same results. It is, therefore, clear that after right lateral hemisection of the cat's spinal cord at the level of the first lumbar segment the depressor reactions obtained from the brachial and right sciatic nerves are normal while those obtained from the left sciatic nerve are greatly reduced. This would indicate that the conduction in the spinal cord of the afferent impulses producing the depressor reflexes is chiefly contralateral but to some extent also homolateral.

The changes in the pressor reflexes were not so clear. In every case the pressor reaction obtained from strong sciatic stimulation, is below the average pressor response obtained by stimulating the sciatic in normal cats with the same strength of current, Table III. With one

exception the pressor response from either sciatic was considerably less than from the brachial. Since the pressor reflexes from both sciatic nerves are decreased after lateral hemisection it would seem that the afferent impulses bringing about this rise in blood pressure must pass up the cord bilaterally.

It will be noted further that the rise from right sciatic stimulation is less than that from stimulation of the left sciatic. This may be due to the impulses passing up the cord somewhat better homolaterally than contralaterally. Or it may be due to the fact that the antagonistic depressor reflex is almost eliminated from the left side, while on the right side the depressor reflex is normal and tends to overpower the weakened pressor. It seems probable, therefore, that the afferent impulses producing a rise in blood pressure are conducted bilaterally in the cord, and either equally well on both sides or somewhat better homolaterally.

Since the best depressor reactions were obtained from the sciatic on the side of the lesion and the best pressor reactions from the sciatic on the side opposite the lesion, it is clear that the afferent paths in the cord involved in these two reflexes are not the same.

POSTERIOR HEMISECTION

A posterior hemisection was performed on six cats at the level of the first or second lumbar segments. The autopsy, performed 6 to 84 days later, showed that in two (A 1 and A 2) the lesion was in the first, in three (O 2, O 3 and A 3) in the second, and in one (O 4) in the third lumbar segment. Microscopical examinations showed that in each case the lesion involved all of the posterior funiculus, the posterior part of the lateral funiculus and all of the gray matter except the anterior horns (fig. 4). In cat O 2 the entire gray substance was destroyed at the level of the lesion.

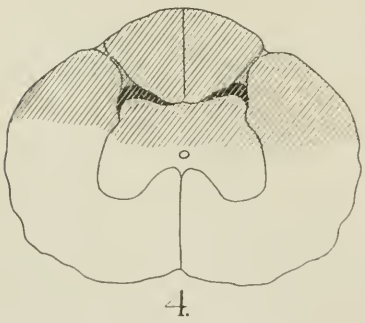


Fig. 4. Diagram of the second lumbar segment of cat A 3. The shaded area shows the part involved in the posterior hemisection.

So far as could be determined by careful tests these cats felt pain equally well in all four extremities. These lesions in the posterior half of the cord had had no appreciable effect on the conduction of

pain impulses from the hind limbs to the cortex. This is in keeping with the results of most other recorded experiments which place the pain path in the anterior part of the lateral funiculus. The interest in these experiments lies in the fact that they show, contrary to the assumption on which this investigation was started, that the apex of the posterior horn is at least not the chiefh of pat pain toward the cerebral cortex. It is also of interest to note that in cat O 2 the gray matter was completely destroyed at the level of the lesion, showing that pain is not transmitted upward in the gray matter of the cord.

The vasomotor reactions on these cats were tested from 6 to 84 days after the operation. It was found that in each animal stimulation

TABLE IV

Reflex changes in blood pressure after posterior hemisection

CAT	INITIAL BLOOD PRESSURE	POSITION OF SECONDARY COIL	CHANGES IN BLOOD PRESSURE IN MM. HG.		
			Brachial	Right sciatic	Left sciatic
0–2	138	4.5	$+18^{2}$	-2^{5}	-2^{5}
0–3	152	4.5	$+31^{2}$	$+3^{2}$	$+3^{2}$
0–4	148	4.5	+29	+15	+15
A–1	148	6.0	$+21^{2}$	-1^{2}	-13^{2}
A–2	160	8.0	$+16^{2}$	0^{2}	$+4^{4}$
A–2	160	0.0	+20	−3	−2
A–3	104	6.0	+14	−8	−6
A–3	104	4.0	+12	−8	−8

+ indicates a rise.

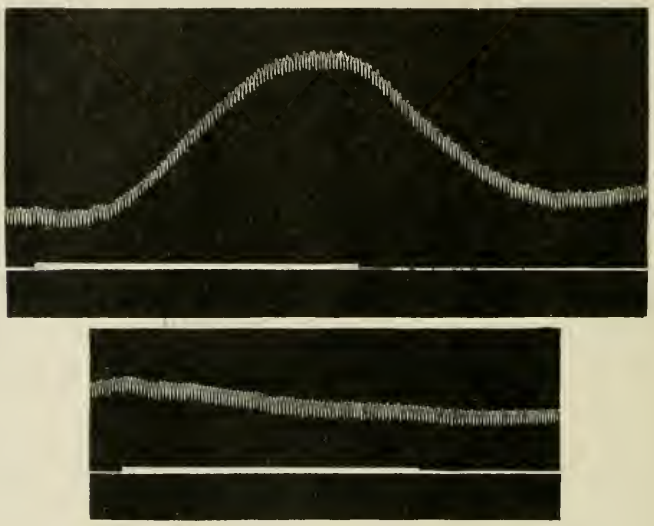
− indicates a fall.

Small figures indicate the number of individual tests which have been averaged.

of the brachial nerves with strong currents (s.c. 0 to 8) gave normal pressor responses varying in height from 12 to 31 mm. Hg. (Table IV). With the same stimulation little or no response was obtained from either sciatic (fig. 5). The slight reaction obtained was more often a drop than a rise. In one cat a rise of 15 mm. Hg. was obtained from sciatic stimulation, but in this animal we had failed to tie the legs securely to the animal board and it is possible that flexion of the thigh on the abdomen may have caused this rise by increasing intra-abdominal pressure. Leaving this result to be explained, the other five cats showed a negligible rise of 3 to 4 mm. Hg. or a drop of 2 to 8 mm. Hg. on sciatic stimulation with currents that gave strong pressor reactions from the brachial nerves. It is thus clear that the nociceptive impulses which

produce a reflex rise in blood pressure travel up the posterior half of the spinal cord along paths which are not the same as those taken by the nociceptive impulses toward the cortical centers for conscious pain.

No systematic attempt was made in these cats to develop the depressor reflex with weak stimulation. Such tests, as were made, indicate that the depressor mechanism was either normal or somewhat



5.

Fig. 5. Blood pressure tracings from cat O 3 with posterior hemisection of the cord. Base line raised 57 mm. Upper tracing, rise of 42 mm. Hg. on stimulation of the left ulnar with strong faradic current *s.c.* 4½. Lower tracing, slight drop on stimulation of the right sciatic with strong faradic current *s.c.* 4½.

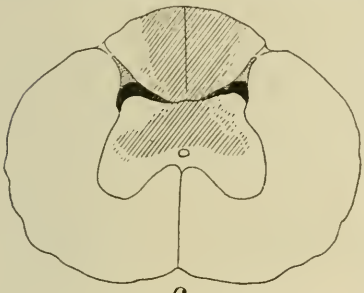
reduced in activity. No exaggerated depressor reactions were obtained in this series.

This question now suggests itself: What part of the posterior half of the cord is involved in this pressor vasomotor reflex? The following paragraphs will show that it is not the posterior funiculus.

SECTION OF THE POSTERIOR FUNICULUS

At the level of the second lumbar segment the medial three-fourths of the posterior funiculus contains all of the dorsal root fibers of the lower lumbar and sacral roots which have reached this height in the

cord. The lateral one-fourth at this level contains only fibers from the second and third lumbar nerves. Hence by cutting the medial three-fourths of the posterior funiculus in the second lumbar segment, as was done in cat O 9, it is possible to cut all the fibers in this funiculus associated with the sciatic nerve and yet run no risk of injuring the apex of the posterior horn. This was attempted in two cats. In cat O 10, the apex of the posterior horn was damaged on one side and the vasomotor reactions showed evidences of this. But in cat O 9 a satisfactory lesion was obtained, although in this cat in addition to a section of the medial three-fourths of the posterior funiculus considerable damage was done to the gray matter around the central canal (fig. 6).



6.

Fig. 6. Diagram of the second lumbar segment of cat O 9. Shaded area shows the part involved in the section of the medial three-fourths of the posterior funiculus.

As was to be expected from the results on posterior hemisection both of these cats showed no hypalgesia in either hind limb. The vasomotor reactions in cat O 9 were entirely normal (Table V). Since in this cat the impulses producing both the pressor and the depressor reactions traveled up the cord in a normal manner it is clear that the posterior funiculus takes no part in the conduction of the nociceptive impulses to the vasomotor centers.

TABLE V

Reflex changes in blood pressure after section of the posterior funiculus—Cat 0–9

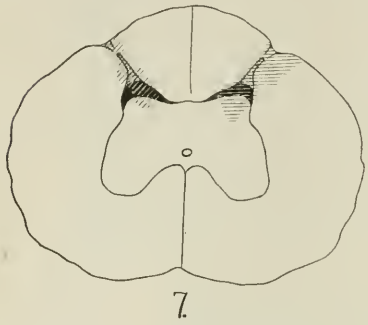
INITIAL BLOOD PRESSURE	POSITION OF SECONDARY COIL	REFLEX CHANGE IN BLOOD PRESSURE IN MM. HG.		
		Brachial	Right sciatic	Left sciatic
142.....	16	−14	−10	−27
	6	+31	+28	+20

− indicates a fall.
+ indicates a rise.

BILATERAL LESIONS IN THE APICES OF THE POSTERIOR HORNS

In the apex of the posterior horn is situated the tract of Lissauer and the substantia gelatinosa Rolandi. These structures are associated with the dorsal roots and belong on the afferent side of the nervous system. But their special function has remained unknown.

Six cats were operated on with the idea of destroying the apex of the posterior horn on both sides at the level of the first lumbar vertebra. The autopsies showed the lesion in each case located in this segment, but microscopical examination showed that the lesion was fairly complete in only two cats A 6 and A 8 (fig. 7). In each of the other cats the destruction of the apex of the posterior horn was incomplete on one or both sides. In cat A 11 the tract of Lissauer was destroyed on both sides and a little of the substantia gelatinosa on one side. In cats A 7, A 10, and A 12 the tract of Lissauer was entirely destroyed on one side and more or less intact on the other. In these three cats the substantia gelatinosa suffered but little injury on either side. In cats A 6, A 8, and A 11 the bilateral destruction of the apex of the posterior horn was most complete and they gave the most characteristic vasomotor reactions.



7.

Fig. 7. Diagram of the first lumbar segment of the cord of cat A 8. Shaded area shows the part involved in the bilateral lesions of the apices of the posterior horns.

As was to be expected from the results of posterior hemisection none of these six cats showed any hypalgesia of the hind limbs. These animals tested from 11 to 23 days after the operation showed a very remarkable variation from the normal vasomotor reaction, Table VI. With weak stimulation (s.c. 16 or 17) normal depressor reactions were obtained from both sciatics and the brachial nerves. With increasing strengths of current the reflex drop became greater and greater. With normal cats the greatest drop was obtained with weak or moderate stimulation (s.c. 17 to 12) and as the current was increased beyond this optimum depressor stimulus the drop became smaller and soon gave place to a rise. The greatest drop obtained from a normal cat was 28 mm. Hg. In these cats with lesions in the apices of the posterior horn the drop continued to increase as the strength of the stimulus was

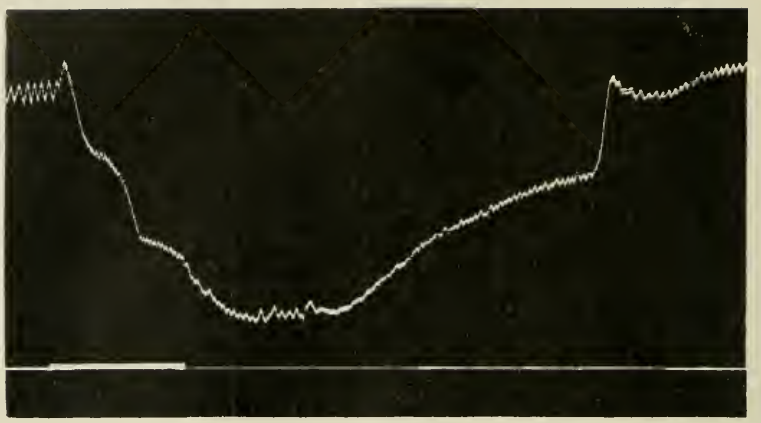
TABLE VI

Reflex changes in blood pressure after bilateral lesions in the apices of the posterior horns

CAT	INITIAL BLOOD PRESSURE	POSITION OF SECONDARY COIL	CHANGES IN BLOOD PRESSURE IN MM. HG.		
			Brachial	Right sciatic	Left sciatic
A–6	158	17	− 5	−11	− 4
		12	−12	−23	−10
		8	−46	−47	−35
		4	−29	−40	−26
A–7	136	16	− 9	− 8	0
		*4	+12	+10	+11
			−44	−32	−30
A–8	140	17	−11	−13	−12
		4	−44	−52	−42
A–10	136	17	−20	− 6	−10
		9	−21	−26	−15
		8	− 4	−11	
		*6	+28	+16	+25
			−38	−38	−31
A–11	138	17	−15	−18	−14
		12	−20	−43	−35
		9	−33	−44	−31
		6	−60	−58	−36
A–12	130	17	−10	−22	− 4
		9	−20	−28	−27
		8	−11	−27	−21
		6	+43	+28	+26

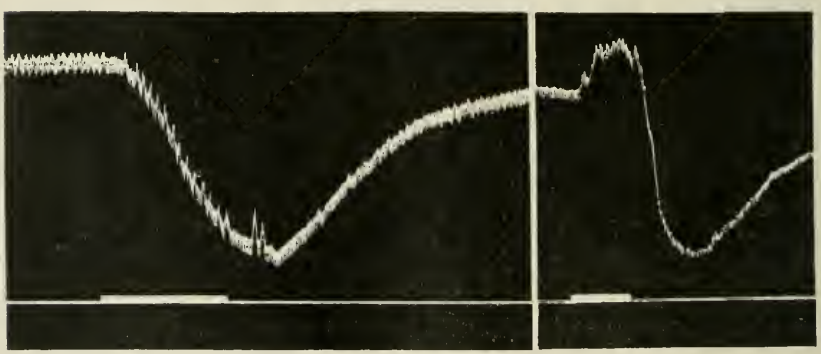
\+ indicates a rise.
− indicates a fall.
* A–7 and A–10 show rise followed by marked drop.

increased until with strong stimuli (s.c. 4 to 9) which would normally produce strong pressor responses the fall in blood pressure became two or three times as great as any that was obtained from normal cats (figs. 8, 9, 10). From the right sciatic a drop of 40 mm. Hg. was obtained with cat A 6, a drop of 52 mm. Hg. with cat A 8, and one of 58 mm. Hg. with cat A 11. It should be noted that these reactions are two or three times as great as can be obtained by stimulating the same nerves in normal cats and were obtained by strength of stimulation



8.

Fig. 8. Blood pressure tracing from cat A 11, with bilateral lesions in the apices of the posterior horns. Base line raised 26 mm. Drop of 58 mm. Hg. on stimulation of the right sciatic with strong faradic current *s.c. 6*.



9 10.

Fig. 9. Blood pressure tracing from cat A 8 with bilateral lesions in the apices of the posterior horns. Base line raised 33 mm. Drop of 52 mm. Hg. on stimulation of the right sciatic nerve with strong faradic current *s.c. 4*.

Fig. 10. Blood pressure tracing from cat A 7 with bilateral lesions, in the apices of the posterior horns. Base line raised 42 mm. Slight rise followed by drop of 44 mm. Hg. on stimulation of the left brachial nerves with strong faradic current *s.c.4*.

which would normally produce the greatest pressor reaction. No pressor responses could be obtained from these three cats. It will be noted that in all these cats the brachial nerves gave the same response as the sciatics. This is difficult to explain but an attempt at an explanation will be made later.

The cats in which the tract of Lissauer was not completely destroyed on both sides (A 7, A 10, A 12) do not give clear cut results (Table VI); but each shows a tendency for the depressor reaction to predominate. In cats A 7 and A 10 strong stimulation (s.c. 4 to 6) gave a moderate rise in blood pressure followed by a much greater fall. The character of such a reaction is shown in figure 10. The drop carried the blood pressure two or three times as far below the original level as the rise carried it above that level.

The abruptness of the drop in cats A 7, A 10, and A 11 may indicate that vagus inhibition of the heart plays an important part in this reaction. Yet this cannot be the chief cause of the fall in blood pressure since in cats A 6 and A 8 section of both vagi did not affect the reaction. In fact the tracing from cat A 8 (fig. 9) was taken after both vagi had been divided.

In cat A 12 the lesions in the tracts of Lissauer were not complete bilaterally. Well marked pressor responses were obtained from sciatic stimulation but a greater one was obtained from the brachial. The results would be easily explained on the assumption that the pressor impulses pass up in the tract of Lissauer and that the partial lesions of these tracts in cat A 12 had partially shut off these impulses arising in the sciatics from reaching the vasomotor centers. In fact the results from cat A 12, though unlike those from the other five of the series are more easily explained than the others.

The question arises, why were not pressor responses obtained from the brachial nerves in the other cats of this series? Since the brachial nerves are connected with the cord above the level of the lesion and the lesion was below the segments from which most if not all of the vasomotor fibers leave the cord, it is difficult to understand why the vasomotor responses from the brachial nerves should have been altered at all. One possible explanation would be that the cutting off of the impulses normally ascending in the apex of the posterior horn from all of the nerves below the first lumbar so lowered the tone of the series of short relays by which the impulses are transmitted that the pathway may become less conductive than normal even to impulses from the brachial nerves. But this explanation is not altogether

satisfactory and the most we can say at present is that lesions of the apices of the posterior horns in upper lumbar segments of the cord cause a complete reversal of the vasomotor responses from strong stimulation of the spinal nerves. Other experiments along this line are in progress.

EFFECT OF CORD LESIONS ON REFLEX CHANGES IN RESPIRATION

In the six cats with bilateral lesions of the apices of the posterior horns, stimulation of the sciatic caused the same increase in rate and depth of respiration as in normal cats. Since the pressor responses were eliminated in five of these cats it would seem that the afferent paths to the respiratory centers are not the same as those to the pressor vasomotor centers. Section of the medial three-fourths of the posterior funiculus also had no effect on reflex changes in respiration. We conclude that the path to the respiratory center is neither in the posterior funiculus nor in the apex of the posterior horn.

In the six cases of posterior hemisection the reflex changes in respiration from sciatic stimulation were greatly reduced though not entirely eliminated. This indicates that the impulses leading to reflex changes in respiration pass up in part at least in the posterior half of the lateral funiculus.

RELATION OF PAIN TO THE VASOMOTOR REFLEXES

All of the cats whose vasomotor reflexes were tested felt pain equally well in all four extremities. So far as could be determined none of them showed any hypalgesia. The complete elimination of the pressor reflex in many of these cats shows that within the cord the pressor path and the path for conscious pain are not the same. On the other hand the depressor reflex was not eliminated in any of these cats. This path was shown to be chiefly crossed and is probably located in the lateral funiculus. Although we could not demonstrate that the pain path was chiefly crossed in cats this has been shown to be the case in most other mammals and in man. It may be that the more accurate blood pressure tracings showed a difference in the two sides which the more crude pain tests failed to show. It is therefore possible that the afferent impulses which are felt as pain and those which produce the depressor vasomotor reactions travel the same paths in the spinal cord.

THEORETICAL CONSIDERATIONS

1. Vasomotor reflex arcs. It is difficult at first to see how the alterations in the vasomotor reflexes which we have described can be accounted for. It is possible however to formulate a conception of the vasomotor reflexes which would explain all our observations. This we present in a purely tentative way as a working hypothesis.

It has been shown by Pike (20) and Sherrington (10) that after section of the cervical cord and during cerebral anaemia the vascular tone and pressor reflexes, which are at first lost, quickly return. This in our mind throws considerable doubt on the importance of the "chief" vasoconstrictor or pressor center in the medulla and in our theory we ignore the brain entirely, although nothing in our theory would be inconsistent with the afferent paths passing by way of the medulla. It is well known that the vasomotor impulses ultimately leave the cord by way of the thoracic and upper lumbar spinal nerves. We shall consider the paths by which impulses from the sciatic ascend to the vasomotor centers without committing ourselves as to the location of these centers.

We assume that in the vasomotor reflexes there are two separate paths from the sciatic nerve to the efferent vasomotor neurones; and for brevity we will speak of a pressor path and a depressor path according to the vascular responses produced by the impulses which they carry. That these are separate paths seems to be true, since after lesions of the spinal cord one type of reflex may be decreased or destroyed while the other is normal or augmented.

The pressor path, according to this hypothesis, is in the apex of the posterior horn and is either equally bilateral or chiefly homolateral. This would account for the loss of the pressor reflex from sciatic stimulation after posterior hemisection and after bilateral injury to the apices of the posterior horns.

The depressor path is not involved in section of the posterior funiculus nor in bilateral injury to the apices of the posterior horn. It must therefore be ventrolateral to these structures probably in the lateral funiculus. Some damage seems to have been done in this path to posterior hemisection but as to this our results are not clear. The results obtained on cats with lateral hemisections showed that the depressor path was chiefly crossed.

The impulses traveling along the two paths are mutually antagonistic and in normal animals vasomotor reactions represent a balance between

them. The depressor path has a low threshold; and by it weak stimuli are able to reach the efferent vasomotor neurones. The pressor path has a high threshold since it is composed of the short fibers and many synapses of the apex of the posterior horn. With weak stimuli the impulses pass up the depressor path only, resulting in a drop in blood pressure. With stimuli of medium intensity they pass up both paths; stimulation and inhibition balance each other with little or no change in blood pressure. With strong stimuli they still pass up both paths but the strong pressor overcomes the depressor impulses, resulting in vasoconstriction and increased blood pressure.

After bilateral lesion of the apices of the posterior horn the pressor path is so impaired that with strong stimulation the inhibitory impulses along the depressor path predominate and produce marked depression. This would account for the marked depression obtained in this series from sciatic stimulation. But similar depressions were obtained from the brachial nerves in this set of experiments, although no cord lesion was interposed between the origin of these nerves and the vasomotor efferent neurones in the thoracic cord. In order to explain this result it is necessary to assume that the normally high resistance of the pressor path has been increased above the lesion by the cutting off of the impulses normally ascending in this path from the lower half of the body.

2. The intraspinal conduction of nociceptive afferent impulses. It is generally admitted that painful afferent impulses are associated with uncontrollable nervous discharge through the autonomic system, one expression of which is the vasomotor reflex (Sherrington in Schaffer's *Text of Physiology*). Obviously only such part of these impulses as ascend to the cortex are responsible for the production of pain. Hence "nociceptive" is a better general term than "painful" and includes the afferent impulses producing the pain reflexes as well as those producing conscious pain. The apex of the posterior horn is involved in the conduction of the nociceptive impulses producing reflex vasoconstriction but not in the upward conduction of those which are felt as pain. It seems probable to us that the tract of Lissauer and the substantia gelatinosa which are well developed in all vertebrates form the primitive mechanism for the reception and intersegmental conduction of nociceptive afferent impulses. In lower vertebrates there is no spinothalamic path for the conduction of painful afferent impulses toward the cerebrum, but these lower forms must have some mechanism for the reception and intersegmental conduction of nociceptive impulses. The tract of Lissauer and the substantia gelatinosa are present

in well developed form in these lower vertebrates and their structure is such that they are especially adapted for intersegmental conduction. Even in the mammal they retain the function of conduction of some nociceptive impulses as is evidenced by their relation to the pressor reflex. With the development of the cerebral cortex in the higher vertebrates this mechanism in the apex of the posterior horn would still retain, according to this theory, its function of reception of all nociceptive impulses. Connected with it there would be developed a new path-way to the cerebral cortex, the spinothalamic, which would carry the pain impulses from the primitive nociceptive mechanism to the cortical centers for conscious pain..

CONCLUSIONS

1. Lateral hemisection in the upper part of the lumbar cord results in a great reduction of the depressor reaction obtained from stimulation of the sciatic on the side opposite the lesion. The depressor reactions from the sciatic on the side of the lesion and from the brachial nerves are normal. There is considerable reduction in the pressor reactions from both sciatics, the greater reduction being in the pressor reactions from the sciatic on the side of the lesion.

2. Posterior hemisection in the upper part of the lumbar cord almost entirely obliterates the pressor reflex from sciatic stimulation but has less effect on the depressor reflex.

3. Section of the posterior funiculus in the lumbar region is without influence on the vasomotor reflexes.

4. Bilateral lesions in the apices of the posterior horns obliterates the pressor reflex. The depressor reflex from weak stimulation is normal but becomes greatly exaggerated when strong stimuli are used. This amounts to a complete reversal of the normal reaction. In this group of experiments the same results were obtained from brachial and sciatic stimulation.

5. A comparison of the effects of the same lesions on the conduction of pain and on the reflex changes in respiration and blood pressure shows that the afferent spinal path involved in the pressor reflex cannot be the same as the path for conscious pain nor the same as the afferent path to the respiratory center. On the other hand it is possible that the afferent spinal path involved in the depressor reflex is the same as that involved in reflex changes in respiration, and in the conduction of pain.

6. None of the lesions (lateral hemisection, posterior hemisection, section of the posterior funiculus, destruction of the apices of the posterior horn) had any noticeable effect on the conduction of pain in these cats.

7. It seems probable that there are two separate afferent spinal paths involved in the vasomotor reflexes: the pressor path, equally bilateral or chiefly homolateral in the apices of the posterior horn; and the depressor path chiefly crossed and located in the lateral funiculus.

It is a pleasure to acknowledge our indebtedness to Professors McGuigan and Hoskins for many helpful suggestions which we have received from them during the progress of this investigation.

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RHYTHMICAL CONTRACTION OF THE SKELETAL MUSCLE TISSUE OBSERVED IN TISSUE CULTURES

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The following observations upon the skeletal muscles of chick embryos in tissue cultures show that isolated fibers and myoblasts possess the property of automatic, rhythmical contractility, in the medium employed.

Among a number of preparations of myotomes of the tadpole explanted into frog lymph, Harrison (3) noticed, in a few instances when the myotome was thin, the differentiation within the myotome of the primitive myoblast into a cross striated fiber. He states that such fibers do not contract unless a part of the neural canal remains attached to the explanted myotome. This result of Harrison may be due to the fact that lymph was used as a medium in consequence of which the necessary salts to stimulate the muscle fiber may not have been present, or it may have been due to the fact that Harrison was dealing only with the myoblast within the thick explanted myotome and not with myoblasts and muscle fibers, which had grown out into the surrounding medium. The following observations show that in the case of the chick skeletal muscle, it may contract rhythmically when no nerve tissue is present. It is interesting to note that Harrison observed the primitive myoblast differentiate into the striated muscle fiber, since Champy (2) finds that the striated muscle fiber of the rabbit, when explanted into plasma, dedifferentiates into an indifferent mass of cells, through which the scattered remains of the myofibrillae can be observed. Champy does not state whether the muscle tissue contracted rhythmically at any time during this process.

Burrows (1), described the rhythmical contraction of an isolated heart muscle cell at the periphery of the growth of a tissue culture in a plasma medium. Burrows states also that a group of heart muscle cells, which had divided and differentiated from cells of the explanted piece, but which had become entirely separated from the other growth and

also from the explanted piece, continued to beat rhythmically, although with a changed rhythm from that of the old piece. The explanted piece of embryo chick heart may contain ganglion cells from which nerve fibers may grow, but such nerve fibers are so easily distinguished among or over the cells of the new growth, that it is possible to determine at a glance whether the preparation contains nerve fibers or not. In the case of the isolated heart muscle cell, which Burrows observed in rhythmical contraction, it is needless to state, there was no possibility of nervous influence. These observations of Burrows determine that it is possible for a heart muscle cell to contract rhythmically when entirely free from the nervous system and subject only to the stimulus of the surrounding plasma medium.

Rhythmical contraction of muscle tissue independent of nerve influence has been demonstrated by means of numerous physiological experiments among which some of the well known observations are those of Howell, Loeb, Stiles and Lingle.

Howell (4) found that

> a strip of vena cava from the heart of the terrapin may be kept in continuous rhythmic pulsation during forty-eight hours or more when immersed in baths containing only the inorganic salts, sodium chloride, potassium chloride and calcium chloride. Under normal conditions the stimulus that leads to a heart contraction is dependent upon the presence of calcium compounds in the liquids of the heart, but for rhythmic contractions and relaxations a certain proportion of potassium compounds is also necessary. The sodium chloride seems to be essential only in preserving the osmotic relations between the tissue and the surrounding liquid.

Loeb (9), observed that the skeletal muscle of a frog contracted rhythmically when placed in a 0.7 per cent solution of sodium chloride. Loeb concluded from his observations that it is only the antagonistic action of the calcium and magnesium salts of the blood, which prevent the skeletal muscles from contracting rhythmically.

> The solutions of Na-salts produce rhythmical contractions only if the muscle cells contain Ca-ions in sufficient number. As soon as there is a lack of Ca-ions in the tissue the Na-ions are no longer able to cause rhythmical contractions. On the other hand, if we add Ca-salts in sufficient quantity to the NaCl solution, it will no longer cause rhythmical contraction in a fresh muscle of the frog. It therefore looks as if the presence of a certain quantity of Na-ions caused contractions, but if the quantity of the Na-ions becomes too great in proportion to the Ca-ions, the muscle loses its irritability. On the other hand if there are too many Ca-ions present the rhythmical contractions become also impossible.—Loeb.

Stiles (10) showed that rhythmic contraction can be set up in the smooth muscle of the oesophagus of the frog. His conclusions correspond more with Howell's than with those of Loeb, i.e., he finds that the calcium salts have a direct action in the stimulation of the smooth muscle cells to contraction.

While a strip of smooth muscle in a 0.7 per cent solution of sodium chloride may contract irregularly for about an hour, in a Ringer's solution it will contract rhythmically for many hours. When the strip comes to rest in Ringer's solution, it can be again stimulated by successive quantities of calcium until the calcium chloride reaches 0.1 per cent when the calcium becomes depressive if not toxic. The calcium and potassium are both necessary to maintain rhythmic contraction for any length of time, but the sodium is the necessary salt, although the muscle frequently fails to contract at all in the sodium solution until the calcium is added.

Lingle (7) (8), described the rhythmical contractions of the isolated ventricle of a tortoise heart which does not beat in the blood when placed in pure sodium chloride solution. If the ventricle remains in a sodium chloride solution it ceases to beat. The pure solution of sodium chloride acts as a poison. A small amount of calcium however acts antagonistically to the injurious action of the sodium chloride. Calcium cannot start the contraction of the ventricle, but it is necessary to maintain the rhythmical contraction started by the sodium chloride.

The above together with other later work shows that it is possible for any muscle (heart, smooth or skeletal) to contract rhythmically, provided the necessary salts to stimulate the muscle to contraction are present in the tissue itself and in the surrounding medium.

In the following observations tissue cultures were made from pieces of the legs of 4, 5, 6, 7, 8, 9 and 10 day chick embryos, explanted into Locke's solution +0.5 per cent dextrose +10 per cent boullion according to the method of Lewis and Lewis (5) (6).[1]

The growth of cells is rapid and luxuriant, anywhere from one to fifty muscle fibers may have grown out radially from the explanted piece at the end of 24 hours (fig. 1). Also numerous isolated fibers

[1] Most of the failures to obtain growth in tissue cultures in Locke's solution by other observers is probably due to some error in their technic, as I have found it possible to obtain as large a growth in Locke's solution as in plasma. Dr. C. C. Macklin has shown that certain attempts to obtain growth have proven unsuccessful because evaporation of the medium has caused too great a concentration of the medium (unpublished).

and many primitive myoblasts are scattered throughout the new growth. There is an abundant growth of connective tissue and primitive mesenchyme cells. These cells are larger than the myoblasts and contain a larger nucleus. They extend further away from the explanted piece than most of the muscle cells. The connective tissue cells divide rapidly by mitosis and in some cultures the greater number of the connective tissue cells are in some phase of mitosis. Amitosis has never been observed in these cultures but mitotic figures are to be seen on all sides, frequently as many as one mitotic figure to every four or five resting cells. An attempt has been made to count the mitotic figures present in the growth at a given time and frequently more than 100 such figures have been present in one preparation at the same time.

Numerous myoblasts are always present in the growth which develops from an explanted piece of embryo chick leg of any of the above ages (4, 5, 6, 7, 8, 9 and 10 days) and the greater number of the fibers are less differentiated than is normal for a chick embryo of the age from which the explantation was made. Certain cultures have been kept in a healthy condition (many mitotic figures) for 10 and 12 days by frequently washing with the culture medium and in these cultures the muscle fibers redifferentiate somewhat, but a typical striated muscle fiber has never been observed in any growth, not even in that from a more adult chick embryo.

Nerve fibers do not grow out from the explanted piece of chick embryo leg and a nerve fiber has never been observed in any of the tissue cultures used for this study of skeletal muscles.

Mitotic figures are not present in the multinucleated muscle fibers (fig. 2) but the isolated myoblasts which contain a single nucleus frequently divide by mitosis. This division results in two typical myoblasts and not in an indifferent tissue such as that described by Champy.

The muscle fiber has the appearance of a gigantic cell with from 2 or 3 to as many as 60 or more nuclei in a spread out protoplasmic end of the muscle fiber (fig. 2). In a few instances such a protoplasmic end has split up into numerous myoblasts with each a single nucleus. These myoblasts migrate away and divide by mitosis.

A description of the growth and differentiation of the skeletal muscle in tissue culture will appear in a separate paper.

Fig. 1

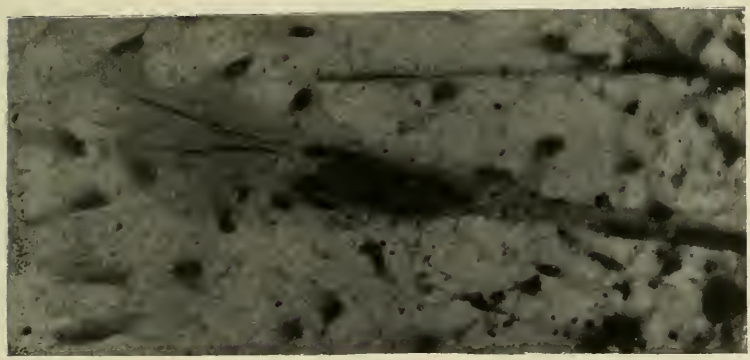

Fig. 2

Fig. 1. Muscle fibers and connective tissue of a 72-hour growth from an 8-day chick embryo wing in Locke's solution + 0.5 per cent dextrose + trace of yolk. Zenker fixation. Mallory stain. Oc. 4. Obj. 16.

Fig. 2. Protoplasmic end of a single muscle fiber, which contains many nuclei and primitive myofibrillae. Same culture as figure 1. Oc. 4. Obj. 4.

Fig. 3

Fig. 4

Fig. 3. Isolated muscle fiber, which contracted 120 times a minute for 8 hours. A 56-hour growth from a 9-day chick embryo leg in Locke's solution + 0.5 per cent dextrose + 10 per cent chicken bouillon. Zenker fixation. Mallory stain. Oc. 4. Obj. 4.

Fig. 4. Two living myoblasts, which contracted slowly (cell 1 once in 15 seconds, cell 2 once in 23 seconds) for one-half hour. Five-day growth from a 6-day chick embryo leg in Locke's solution + 0.5 per cent dextrose + 10 per cent chicken bouillon. Oc. 4. Obj. 4.

RHYTHMICAL CONTRACTION

Among the numerous muscle fibers present in the growth in a tissue culture, one may occasionally contract rhythmically. The time interval of the rhythmical contraction has been different for each muscle fiber as well as for each myoblast that has so far been observed to contract. Some fibers contract very rapidly, i.e., as often as 120 times a minute; others contract once in 2 or 3 seconds; others not oftener than once in 15 or 20 seconds; and some contract very infrequently, i.e., only as often as once in from 1 to 5 or 10 minutes.

Figure 3 is a photograph of an isolated multinuclear muscle fiber, which was not only free from the explanted piece, but also free from other muscle fibers. This fiber was first observed in rhythmical contraction when the culture was 48 hours old and it continued in rhythmical contraction (120 times a minute) throughout the day, at the end of which time it was fixed and stained. The stain used was Mallory's connective tissue stain and the primitive myofibrillae can be seen to extend from one end of the fiber to the other. The growth was from the leg of a 9–day chick embryo and was 56 hours old when fixed. The muscle fibers of the explanted piece were without doubt striated at the time of explantation and therefore it seems reasonable to suppose that this fiber must be either the result of dedifferentiation of the striated muscle fiber (Champy) or of the differentiation of a myoblast.

Figure 4 is a drawing of two living myoblasts during the resting period of a rhythimcal contraction which continued for over half an hour after it was discovered in a 5 days' growth from an explanted piece of a 6-day chick embryo. The time interval of contraction was different for the two cells. Cell 1 contracted every 15 seconds and cell 2 every 23 seconds.

Some well developed muscle fibers with a few cross striated myofibrillae have been observed to contract, but more frequently the property of rhythmical contraction is exhibited by less well developed muscle fibers such as figures 2 and 3 or by the isolated myoblast itself.

The ability of the muscle fiber to contract is not determined by the age of the culture for rhythmical contraction has been observed in cultures from 24 hours to 5 days old. Also the property of contractility is never general for any one culture, i.e., while one or two myoblasts

or muscle fibers of a given culture may contract, the other 50 or more fibers appear inactive or else the time interval of contraction is so slow that it is not observed.

EXPERIMENTAL WORK

An attempt has been made to stimulate the muscle fibers to contract by a change in their environment such as has been shown to stimulate rhythmical contractions by Howell, Loeb, Stiles and others.

The cover slip on the underside of which was the growth to be experimented upon was removed from the vaseline ring and a drop of a different solution at 39° C. was placed upon the growth. The cover slip was then replaced on a hollow ground slide and the growth was studied under the microscope in a warm chamber with the following results.

Sodium chloride. A drop of 0.9 per cent solution of NaCl in distilled water placed upon the growth in a few instances resulted in the immediate contraction of one or more of the muscle fibers. This contraction was repeated a few times and then ceased and did not occur again. In no case did the solution of NaCl stimulate a rhythmical contraction of all the muscle fibers or cells of the new growth. The muscle cell or fiber, which had previously been contracting, continued to contract for a short time and then ceased. It is possible that the NaCl penetrated the extremely thin growth very rapidly and had a toxic action upon the cells. If the NaCl solution was replaced by one which contained $CaCl_2$ as well as NaCl no change occurred. When the growth was again bathed in the medium, which had been used for the explantation (Locke's sol. + 0.5 per cent dex. + 10 per cent bouillon) the growth sometimes recovered and mitosis continued, but more frequently the growth became degenerate.

Calcium chloride. A drop of .025 per cent $CaCl_2$ resulted in the degeneration of the muscle fibers and other cells. A drop of Locke's solution had no effect, i.e., the muscle fiber continued to contract but the inactive fibers were not stimulated to contract. A drop of Locke's solution, which contained an increased amount of $CaCl_2$ either had no effect or resulted in the degeneration of the growth.

Dilution of the medium. A slight dilution of the medium used for the explantation had no definite action upon the contractility of the muscle tissue. A dilution of 10 per cent or more frequently stimulated certain of the muscle fibers and cells to contract, although it also resulted in the degeneration of many of the muscle fibers. The degeneration of the muscle fibers in this case was quite different from that

which usually takes place due to other causes. In this case the entire muscle fiber flowed from one extremity to the other and left no trace of its previous elongated structure. The mass of coarsely granular protoplasm which resulted from the rounding up of the muscle fiber later disintegrated.

Although the method used to stimulate the muscle tissue in these tissue culture growths to rhythmical contraction have so far not been successful, due probably to the fact that the method used was not sufficiently delicate, nevertheless the fact remains that by some so far unknown change, either in the tissue itself or in the surrounding medium, the myoblast and also the muscle fiber of the skeletal muscle of the chick embryo possess the property of rhythmical contractility when entirely free from nerve influences and subject only to the stimulus, which emanates from its environment.

It is interesting to note that the muscle cells inherit this property from cell to cell even when several generations of cells have taken place in a simple Locke's solution. The cytoplasm of the myoblast is quite different from that of the surrounding connective tissue cells. It is less thinly spread out and has a different refraction from the other cells.

CONCLUSION

The skeletal muscle tissue which grows out from an explantation of a piece of embryo chick leg into Locke's solution +0.5 per cent dextrose +10 per cent chicken bouillon, may contract rhythmically in the absence of nervous tissue.

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THE PRESERVATION OF THE LIFE OF THE FROG'S EGG AND THE INITIATION OF DEVELOPMENT, BY INCREASE IN PERMEABILITY

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If the frog's egg is removed from the body of the female and placed in water it soon dies and disintegrates. This is true whether the egg is removed from the ovary, the body cavity or the oviduct. Even though the egg were laid in the normal fashion, it would die if not stimulated by a spermatozoon or in some other manner. Bataillon[2] was able to cause unfertilized frog's eggs to segment by pricking them with a needle. But I (1) found that a much more convenient and efficient method of causing them to segment is to stimulate them with the ordinary 110-volt alternating current from carbon or platinum electrodes placed about two inches apart in the water containing the eggs, for about one second. Bataillon thinks the introduction of a foreign protein, such as is used to induce anaphylaxis, is necessary for the development of these eggs. I found the jelly in which the eggs are imbedded to be permeable to hemoglobin, and it is possible to think of protein diffusing in without the needle prick. But eggs removed so carefully that there is no possibility of foreign protein gaining access to them, segment when stimulated electrically. It is certain that no foreign protein is necessary for the beginning segmentation of this egg,

[1] The nephelometer used in this work was bought with a grant from the Research Fund of the Graduate School.

[2] Bataillon: Comptes Rendus de la Société de Biologie, 1911, lxx, 562.

though it may be necessary for its later development. In the leopard frog, Rana pipiens, this segmentation usually stops before the first cytoplasmic division is completed, although nuclear divisions may continue for several days.

The initiation of cleavage in the frog's egg by the simple prick of a needle, which I have observed in American frogs, might be interpreted as increased permeability. Since I have shown that electrical stimulation of muscle causes increased permeability (2), electrical stimulation of these eggs might be supposed to have the same effect. In order to determine this point, I made some preliminary experiments last year, but owing to difficulty in obtaining material, could offer but meagre data to prove the point (3). This year the leopard frogs bred in the laboratory, and furnished abundant material.

A large number of females of the same size, and that were clasped by males, were placed in a tank with two inches of water. As soon as a female deposited her eggs, these were carefully removed to distilled water. Two females were opened and their eggs placed in separate dishes of distilled water, one as a control and the other for electrical stimulation.

In removing the eggs, the female was washed free from sperm, the head cut off, most of the viscera removed and the blood washed out. The distended oviducts were carefully removed and the blood washed out of the vessels in their walls. A hole was made in each oviduct and it was dragged across the floor of a dry glass dish, leaving a trail of adhesive eggs. When distilled water was added the eggs remained adhering to the glass.

In order to guard against slight differences in the masses of eggs, the two oviducts of one female were emptied in separate dishes and one used as an unfertilized control and the other stimulated electrically. The batch of fertilized eggs of another female was cut in two equal parts, and one part placed in a third dish.

The eggs were washed in repeated changes of distilled water for an hour. During this time the gelatinous coverings of the eggs partially swelled. The sperm traverses the jelly and reaches the inseminated eggs in about one hour, and the eggs rotate until their black poles are turned upward. It is now time to stimulate the unfertilized eggs in one dish. Two clean platinum electrodes, about one inch apart are passed along opposite sides of each row of eggs, until all of the eggs in the dish have been stimulated. These eggs now begin to rotate, and some of them are completely rotated in one minute. If the rows of

eggs are slender so that the jelly is uniformly swollen, all of the eggs rotate at about the same time. None of the eggs in the unfertilized control rotate, no matter how much the jelly swells. This is due to the fact that the jelly sticks tight to the egg, whereas when they are stimulated, a fluid is secreted between the egg and the jelly.

The water is now poured or syphoned off of all the eggs and a measured quantity added to each dish. Ordinary distilled water was boiled and redistilled in an automatic quartz apparatus (4) and used in the experiments. At the end of certain intervals of time, the water in each dish is stirred, and 25 cc. removed for analysis of chlorides with the nephelometer. At the end of seven hours all of the water is removed from all of the eggs. An aliquot part of it is evaporated in quartz for ash. Water is added to all of the eggs and another series of determinations made.

The following table gives a sufficient number of the results to indicate their character.

NO. OF OVIDUCTS TO EACH DISH	cc. H_2O	cc. OF A $\frac{1}{100}$ NORMAL CHLORIDE SOL. IN H_2O			GRAMS ASH IN H_2O
		0–3.5 hrs.	0–7 hrs.	7–30 hrs.	0–7 hrs.
1 unfertilized	500	0.70	1.20	0.38	0.005
1 stimulated	500	1.40	2.24	1.22	0.008
1 fertilized	500	1.22	2.20	1.20	0.007
2 unfertilized	1000	1.40	2.40	1.42	0.015
2 stimulated	1000	1.90	3.60	2.40	0.024
2 fertilized	1000	1.80	4.20	2.00	0.023
4 unfertilized	2000	2.80	4.80	3.00	0.031
4 stimulated	2000	4.00	7.60	4.80	0.053
4 fertilized	2000	3.80	7.20	4.20	0.045

The first column shows the number of oviducts used in supplying each dish with eggs, and the second gives the number of cc. H_2O added. In the first 3.5 hours as much chloride diffused out of the eggs as in 23 hours, beginning with the eighth hour. The table gives the total quantity of Cl in the H_2O as calculated from analysis of a 25 cc. sample, and the result may be converted into grams by multiplying by 0.035.

In obtaining the ash, three-fourths of the H_2O was evaporated down in quartz beakers. During the evaporation the foam was skimmed off and transferred to a platinum crucible. When the volume of the water was reduced to a few cc. it was transferred to the crucible. The

ashing was done at as low a temperature as possible by admitting air into the crucible through a small metal tube while it was heated. Some chlorides may have been lost, but the results are comparative. The ash was found to contain about 60 per cent of insoluble salts and 40 per cent of soluble salts. The ash contained Na, Li, K, Ca, Mg, Cl, SO_4 and CO_3, but no phosphates were detected. The metals were detected in the flame spectrum and the spark spectrum (fulgurator). Mg was also detected by means of the displacement of the bands of alkanin. Na was also determined quantitatively as sodium uranyl acetate and as silicofluoride but the quantities were too small to be accurate. SO_4 was precipitated with BaOH, and CO_2 driven out of the dry ash with acid.

The diffusion of salts from the electrically stimulated eggs is about the same as from the fertilized eggs, but nearly double that from the unfertilized eggs. This difference was found to continue for 50 hours, when the determinations were discontinued as the unfertilized control had died.

In making prolonged experiments it is important to change the water frequently in order to keep down the bacteria, which might bind the salts or interfere with the nephelometer reading. In previous years, I have charred and extracted the samples before nephelometric determinations, but think it a bad practice and only necessary in the presence of bacteria or protein. The traces of dissolved glyco-protein from the jelly did not cause a measurable error as a great excess of $AgNO_3$ (5 drops of a saturated solution) was added to the 25 cc. sample, and the reading was taken in one minute, whereas several hours are required for perceptible reduction of silver by these solutions in the intensity of light to which they were exposed. I found it advantageous to use open tubes closed at one end with black rubber stoppers in the nephelometer, in order to avoid the reflection from the bottom of test tubes, but this was not absolutely necessary with the Richards nephelometer, because a part of the large field shows no reflections, and test tubes can be more easily cleaned.

The supposed relation between increased permeability and cleavage has been fully discussed in a previous paper (5) in which I showed that an increase in permeability of the sea urchin's egg was produced by any treatment which causes cleavage.

The fertilized or stimulated frog's egg may live a long time, whereas the unfertilized frog's egg soon dies when placed in water. The question arises whether the increased permeability saves the life of the egg.

In order to decide this we must first consider the effect of water on the egg.

The frog's egg swells when placed in water, and I found that the unfertilized egg swells more than the fertilized or stimulated egg, until the death of the former terminates the physiological comparison. In eggs of the same female, the mean diameter of 23 unfertilized eggs 30 minutes after being placed in water was 1.52 mm. whereas the mean diameter of 23 that had been stimulated by the electric current and left in water 30 minutes was 1.47 mm. After cleavage begins the estimation of the volume of the eggs becomes more difficult, and on the formation of spaces between the cells of the fertilized egg the apparent volume is much greater than the real volume of the protoplasm. But I think that one effect of fertilization is clearly the retardation of the swelling of the egg. One need only suppose that the death of the unfertilized egg is caused by rapid swelling, in order to explain the life-saving action of fertilization or stimulation. Bachman found the unfertilized salamander egg bursts in two to seven hours if placed in water but not if in salt solution. It is not so easy to determine the exact moment of death of the frog's egg.

The retardation of the swelling of the fertilized or stimulated egg is evidently due to the loss of osmotic substances, such as the salts which I have given in the above table. At the moment of increase in permeability, a concentrated solution of these substances passes from the egg and pushes out an adhering membrane which we may call the fertilization membrane. Bialaszewicz[3] claims that a measurable decrease in volume of the egg takes place at this time. We may take this decrease in volume of the egg to equal the volume of the secreted fluid, but after this perivitelline fluid has once been secreted it increases by absorption of water through the fertilization membrane. I found this membrane to be easily permeable to salts since it cannot be easily plasmolyzed (shrunken) by immersion in hypertonic salt solutions. Hence it is probable that the perivitelline fluid contains other less diffusible substances. An analysis of the perivitelline fluid of the Amblystoma egg showed that it contained salts and organic substances but only a trace of protein. The perivitelline fluid of the egg of the giant salamander contained 0.16 per cent of dissolved substances. Backman and Runnstrom[4] suppose these substances to be chiefly secreted by the suckers that develop on the head of the frog embryo,

[3] Bialaszewicz: Bull. Acad. Sc. Cracow, Math.-Nat., Oct. 1908.

[4] Backman and Runnstrom: Pflüger's Archiv, 1912, cxliv, 313.

but this is hardly in harmony with the fact that the salamander embryos have no suckers.

The function of the perivitelline fluid seems to be to make room for rotation of the egg and extension of the embryo, by pushing out the fertilization membrane, which is chiefly effected by the osmotic pressure of the dissolved substances that it contains. The loss of salts must be more or less independent of this, since salts are lost continuously during development, and salts would not be very effective in pushing out the membrane since it is permeable to them.

This loss of salts explains the results of Backman and Runnstrom[5] who found that the osmotic pressure of the frog's egg is reduced when it is fertilized and placed in water. They attempt to explain it, however, by erroneously assuming that fertilization causes a coagulation of the proteins and that the coagulation adsorbs the salts. They seem to consider this egg as a diphasic system in which the watery phase forms the main bulk of the egg. On the contrary, I found the cytoplasm to be a 4-phase system in which the watery phase is a very small fraction of the total volume (6). The salt would have to be lost from this watery phase only, in order to reduce the osmotic pressure of the egg, and the more permeable the plasma membrane is to them the less osmotic pressure they can exert while in the egg. I found the watery phase to be 16 per cent of the whole egg and to contain 82 per cent water (7). These figures are a little too high owing to slight admixture of other phases, but after removal of these and of the dissolved proteins, the water-soluble substances in this layer formed only 1.7 per cent of the weight of the egg.

The chlorides diffusing out of the egg in seven hours would make a solution in the watery phase more concentrated than $\frac{1}{100}$ normal and the total salts diffusing out in this time would form nearly 1 per cent of the watery phase. Since the osmotic pressure of the ovarian egg corresponds to a 0.166 normal solution of NaCl and is probably lowered in the passage of the egg through the oviduct, the increased permeability and diffusion seem to be sufficient to account for the failure of the egg to die in the very hypotonic water in which it develops.

By an analysis of the ovarian eggs, I found that the chlorides were not sufficient to raise the osmotic pressure of the watery phase to equal that of frog's blood or a 0.166 normal NaCl solution, and that they were not bound during coagulation of the protein (furthermore there is

[5] Backman and Runnstrom: Biochem. Zeitsch., 1909, 390.

no evidence that fertilization causes coagulation). About 25 cc. (26.6 g.) of ripe ovarian eggs of the leopard frog were freed from lipoids, dried, powdered and boiled in 200 cc. of distilled water, slightly acidulated with acetic acid in order to coagulate the proteins, and filtered. The ash of the filtrate required 1.55 cc. of $\frac{1}{10}$ normal $AgNO_3$ to precipitate the chlorides, whereas the ash of the coagulum required only 0.2 cc., which might have been due to that part of the filtrate adhering to the coagulum. Since this egg contains 53 per cent water, about seven times as much water was used to dissolve out the chlorides as is contained in the egg, but since the egg is permeable to salts, the whole pond is available to wash out the salts when the egg is laid in natural surroundings.

The supposition that the osmotic pressure of the frog's egg falls while it is in the oviduct is supported by the fact that such a change happens to the bird's egg. Atkins[6] found that the osmotic pressure and chloride content of the egg white is much less than that of the bird's blood. The egg white corresponds roughly to the jelly of the frog's egg, both containing some glyco-protein and being secreted by the oviduct. A coat of jelly of low osmotic pressure would cause a lowering of the osmotic pressure of the egg, provided the protoplasm is at all permeable to salts or water. Bialaszewicz[7] found that the ovarial egg of the hen has an osmotic pressure nearly equal to that of the hen's blood, but that the osmotic pressure of the yolk as it descends the oviduct, steadily decreases. The yolk extracts the water from the white so that the latter becomes denser.

Backman and Runnström state that the osmotic pressure of the fertilized frog's egg is as low as that of the pond water ($\Delta = 0.045$) but they admit that some of the jelly was adhering to the eggs that were used in the cryoscopic determination. Notwithstanding the permeability of the frog's egg it continually swells after the slight shrinkage due to the exit of perivitelline fluid, as I have repeatedly observed. Bialaszewicz made careful measurements of this swelling. Backman[8] observed the same process in the eggs of the toad and the salamander (Triton). Therefore we must conclude that the osmotic pressure of the frog's egg does not fall to the extent that Backman and Runnström suppose.

[6] Atkins: Biochem. Jour., 1909, iv, 480.
[7] Bialaszewicz: Roux's Archiv, 1912, 489.
[8] Backman: Pflüger's Archiv, 1912, cxlviii, 141.

During the development of the frog's egg and tadpole the osmotic pressure rises to become that of the adult frog. This is probably brought about by the solution of the reserve proteins (8) and the formation of osmotic substances, rather than the absorption of salts from without, since the same process takes place in the hen's egg which is cut off from osmotic exchange with the environment. Since the definitive osmotic pressure is maintained fairly constant, Backman and Sundberg[9] suppose that the skin is relatively impermeable to water, from without toward the interior, and that the elastic contraction of the cell walls resist the entrance of water produced by the osmotic pressure. On the contrary the skin of the frog continually absorbs water at a rapid rate (9), and it is only the activity of the kidneys that preserves the frog from dissolution. Any effect of the elasticity of cell walls or the skin, in preventing swelling, must be very slight, since the osmotic pressure is so great (8.9 atmospheres) and the frog will burst if the action of the kidneys is eliminated. The pronephros develops before the egg hatches and the kidney function probably develops before any danger of flooding the tissues with water occurs.

Although swelling may cause the death of the unfertilized eggs, a normal degree of swelling seems necessary for development. The egg contains 46 per cent of solid (dry) materials and the viscocity of the protoplasm is very great. If the viscocity is not reduced by the absorption of water, the segmentation of the cells of the white pole is prevented and the downgrowth of the cells of the black pole retarded. Also by the action of centrifugal force, the solid materials may be packed in the white hemisphere and its cleavage prevented (10). This hard white hemisphere exists in less degree in eggs whose swelling is prevented by immersion in solutions of too high osmotic pressure. For a number of years I have repeated experiments in which the osmotic pressure of the water was raised by the addition of salts. One-tenth molecular solutions of NaCl, and isotonic solutions of chlorides and nitrates of Na, K and Li have the same effect. If the eggs are placed in these solutions immediately after they are fertilized, and allowed to remain 24 hours, the segmentation of the white pole of some of the eggs is prevented. If they remain 48 hours in the solutions a large number are so affected, and if they remain indefinitely none of them develop. The addition of Ca or Mg does not prevent this effect, so it is probably due to osmotic pressure.

[9] Sundberg: Pflüger's Archiv, 1912, cxlvi, 225.

These salts are not entirely without toxic action, for this is manifested in the swelling of serous cavities. The swelling of the pericardium is greater in lithium solutions than in isotonic solutions of Na or K. In more concentrated solutions, the individual cells separate from one another, a condition which Roux called framboisea of the embryo.

A marked contrast exists in the effects of pure salt solutions on the eggs of the frog and those of many fish. These fish eggs are normally impermeable to salts and water and the osmotic effects are never observed. The toxic effects of salts are, however, more manifest (11). These toxic effects, resulting in the swelling of serous cavities and other abnormalities, are associated with increase in permeability (12). Though a slight increase in permeability does no damage, a greater increase causes abnormalities and a still greater increase causes death. It is probable that the swelling of the pericardium of the frog's egg is produced by increase in permeability in the same way as the swelling of the pericardium of the fish egg. The difference between the two is that, whereas the fish egg is normally impermeable to salts and to water, the frog's egg is permeable to water and to a less degree to salts. Fertilization increases this permeability to the optimum degree, and pure salt solutions increase it too much.

SUMMARY

Fertilization or electric stimulation increases the permeability of the frog's egg so that Na, K, Li, Mg, Ca, Cl, SO_4 and CO_3 diffuse out at a faster rate. Probably the swelling of the pericardium, due to the action of pure salt solutions, is caused by too great increase in permeability. Notwithstanding the outward diffusion of salts, the egg absorbs water, and the addition of osmotic substances to the medium, preventing the absorption of water, prevents the segmentation of the white hemisphere, and gastrulation.

The abnormally rapid swelling of the unfertilized egg probably is the cause of its death, and thus the increase in permeability on fertilization lowers the osmotic pressure and saves its life.

The low osmotic pressure of the egg observed by Backman and Runnström was probably caused by three processes: (1) The lowering of the osmotic pressure as the egg descends the oviduct; (2) the increased permeability and loss of salts; (3) the admixture of jelly of low osmotic pressure with the eggs in taking the freezing point.

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(1) McClendon: Science, 1911, xxxiii, 629; and Am. Jour. Physiol., 1912, xxix, 298.
(2) McClendon: Am. Jour. Physiol., 1912, xxix, 302.
(3) McClendon: Science, 1914, xl, 70.
(4) McClendon: Int. Zeit. f. physik.-chem. Biol., 194, i, 31.
(5) McClendon: Am. Jour. Physiol., 1910, xxvii, 240.1
(6) McClendon: Archiv f. Zellforschung, 1910, 386, fig. 1, a.
(7) McClendon: Roux's Archiv, 1909, xxvii, 247.
(8) McClendon: Am. Jour. Physiol., 1909, xxv, 195.
(9) McClendon: Internat. Zeitsch. f. Physik.-chem. Biol., 1914, i, 169.
(10) McClendon: Roux's Archiv, 1909, xxvii, 253.
(11) McClendon: Am. Jour. Physiol., 1912, xxix, 289.
(12) McClendon: Int. Zeit. f. Phys.-chem. Biol., 1914, i, 28.

THE ACTION OF ANESTHETICS IN PREVENTING INCREASE OF CELL PERMEABILITY[1]

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Höber and Lillie have attempted to associate anesthesia with permeability. In a lecture at Woods Hole in 1911, I attempted to express the relation as follows: "If stimulation consists in increase in permeability, we should expect anesthetics to prevent this change."[2]

Most of the attempts at correlating anesthesia and permeability have been indirect. Thus Höber[3] found that anesthetics prevent the action of salts in producing a demarcation current in muscle. Lillie[4] observed that anesthetics prevent the action of salt solutions in causing artificial parthenogenesis and cytolysis of Echinoderm eggs. He also observed that anesthetics prevent the outward diffusion of cell pigment produced by salts, and thinks this a direct proof that anesthetics prevent increase in permeability. Arrhenius and Bubonovic[5] observed that anesthetics retard the hemolytic action of hypotonic solutions. Höber attributes a similar observation to Traube, but I have not as yet found the reference.

It is clear, therefore, that anesthetics in the anesthetizing concentration, tend to prevent the outward diffusion of pigment from pigmented cells. It seemed to me of interest to determine whether non-pigmented substances are affected in this way, especially the salts contained in the cells. In 1911, I had observed that the Fundulus egg is impermeable to salts, but found that Mg came out of the eggs when placed in a slightly toxic solution of NaCl.[6]

[1] This research assisted by a grant from the Research Fund of the Graduate School.

[2] McClendon: Biol. Bull., 1912, xxii, 139.

[3] Höber: Pflüger's Arch., 1907, cxx, 492, 501, 508.

[4] Lillie: This Journal, 1912, xxix, 373, 1912, xxx, 1; 1913, xxxi, 255; Journ. Exper. Zool., 1914, xvi, 591.

[5] Arrhenius and Bubonovic: Meddel. K. Ventensk. Akad. Nobel institut, 1913, ii, no. 32.

[6] McClendon: This Journal, 1912, xxix, 296.

It was later found that various toxic solutions increase the permeability of the egg to salts.[7] The addition of anesthetics to these salt solutions tends to prevent this increase in permeability.[8]

In making these last experiments, Fundulus could not be obtained and the much more delicate eggs of the pike were used. It was found that practically no salts came out of these eggs when they were placed in distilled water. Since the egg contains salts, and it does not swell or burst when placed in distilled water, it must be impermeable to water as well as to salts. When placed in slightly toxic solutions of nitrates or anesthetics the chlorides diffuse out of the egg very rapidly. But when a toxic salt solution ($\frac{1}{10}$ molecular $NaNO_3$) contains an anesthetic in the proper concentration for anesthesia, the chlorides diffuse out of the egg at a slower rate than in the pure salt solution. Thus anesthetics have two effects on these eggs, at a certain concentration they produce anesthesia and at a greater concentration they are toxic. A 3 per cent solution of alcohol or a 0.5 per cent solution of ether produces anesthesia (retards development) whereas a 6 per cent solution of alcohol, or a 2 per cent solution of ether is slightly toxic. Twice as much chloride diffuses out of eggs placed in pure $NaNO_3$ solution as in the same solution containing 3 per cent alcohol. One and a half times as much chloride diffuses out of the egg in the pure $NaNO_3$ solution as in the same solution containing 0.5 per cent ether.

These experiments were extended this year, and the chlorides determined more accurately with a Richards' nephelometer.

MATERIAL

The eggs of the pike (Esox) were used. These eggs are very delicate, and are especially susceptible to changes in temperature, lack of oxygen and to the presence of various substances in the medium. The eggs were shipped from the breeding grounds in northern Minnesota to the State Fish Hatchery in Saint Paul by being spread out in thin layers on stretched muslin in refrigerator boxes. From this point they were brought to the laboratory in a refrigerator box, and those in good condition selected one by one and placed in water that was distilled, boiled and later redistilled in an automatic quartz still.[7] No other water was used in the experiments.

[7] McClendon: Internat. Zeitschr. f. Physik.-chem. Biol., 1914, i, 28.
[8] McClendon: Sci., 1904, xxxviii, 280.

METHODS

The eggs are washed in the redistilled water until the salts practically cease coming out of them, and measured in a series of graduated cylinders of the same bore. That the chlorides have all been washed out of the transparent egg shell follows from the following facts: The last wash waters contain so little chlorides that they cannot be detected with the nephelometer unless the washings are boiled down to a small volume. Tap water usually contains 100 to 10,000 times as much chlorides. The eggs are made permeable to chlorides by $\frac{1}{10}$ molecular $NaNO_3$ solution, and while they remain in this, twice as much chloride diffuses out of the eggs in six hours as in three hours. If these chlorides were held in the thin shell we would expect more to come out in the first three hours than in the second three hours. Since the shell is at all times easily permeable to salts, the source of the chlorides is not the fluid between the egg and the shell, and the small amount of shell substance is probably not the source of such large amounts of chlorides as were obtained from the eggs. I found that when the Fundulus egg is made permeable to chlorides the other salts diffuse out also, and when the frog's egg is made permeable to Cl that SO_4,CO_3, Na, K, Li, Mg and Ca diffused out also. This is probably true of the pike egg also, and enough salts could probably be obtained by diffusion to equal the entire weight of the egg shell, which is protein in composition. The most reasonable hypothesis is that the plasma membrane is practically impermeable to salts (and to water) and that it is made permeable by toxic solutions. The permeable egg may live and develop for several days, showing that the entire store of salts is not necessary for its immediate needs.

EXPERIMENTS

The same volume of eggs was placed in each of a series of Stender dishes, with 30 cc. of the solution to be tried. At the end of six hours, 25 cc. of this solution was removed with a pipette having a trap to prevent contamination with saliva. The sample was transferred to the nephelometer tube, and five drops of a saturated solution of $AgNO_3$ added and mixed. The two matched test tubes used in the nephelometer were filled up to the zero point of the scale by the 25 cc. + 5 drops.

In case any eggs died during an experiment, this experiment was thrown out. Since the material was available in great abundance,

a number of experiments were performed, of which the following are typical:

(1) 5 cc. of eggs in each dish. Chlorides expressed in arbitrary units. Ethyl alcohol expressed in per cent by volume.

Solution.....	$\frac{N}{10}$ $NaNO_3$	+1% alcohol	+2% alc.	+3% alc.	+4% alc.
Chlorides.....	100	60	50	45	60

(2) 15 cc. of eggs in each dish

Solution.....	$\frac{N}{10}$ $NaNO_3$	$\frac{N}{10}$ $NaNO_3$+2% alc.	$\frac{N}{10}$ $NaNO_3$+3% alc.
Chlorides.....	100	50	40

The duration of the experiment, six hours, was selected because this was the maximum duration that could be considered safe. Even then, some of the experiments had to be thrown out because one or two eggs died before they were completed. The $NaNO_3$ has to be pure in order to have the right degree of toxicity. The ethyl alcohol was redistilled over metallic sodium. Although the dishes were covered in all experiments, the experiments with ether were discontinued after it was found that ether has the same effect as alcohol, owing to the fact that some of the ether diffused into the air above the eggs and the concentration of that remaining in the solution was no longer known. It is probable that all anesthetics have the same action.

A word of caution to any one who wishes to make similar experiments on pike eggs: Some of my early experiments were irregular in results. I believe the reason for this is that the egg is made more permeable by increase in temperature,[9] and that different eggs are not affected by the same temperature. I did not determine the maximum temperature at which it is safe to work, but a temperature of about 8° is safe and is easily maintained in a refrigerator. Fundulus eggs may be used at room temperature in Woods Hole, and are preferable in every way. It is clear that 2 to 3 per cent by volume of ethyl alcohol partially inhibits the permeability-increasing action of a pure $NaNO_3$ solution. It is theoretically possible to entirely prevent this increase in permeability if the toxicity of the $NaNO_3$ solution is low enough. The difficulty in demonstrating this lies in the fact that the toxicity of the sodium salt does not depend on its absolute concentration, but

[9] Osterhout: Biochem. Zeitschr., 1914, lxvii, 272.

on the ratio of sodium to calcium. With a very mildly toxic solution of $NaNO_3$ the Ca diffusing out of the egg at the first increase of permeability lowers the toxicity and hence the permeability to such an extent that no permeability increase can be measured by means of the nephelometer, and electric conductivity experiments with their large and numerous sources of error would have to be substituted.

That 2–3 per cent ethyl alcohol is really the anesthetic concentration, follows from the fact that it retards the development of these eggs. The same concentration may not be correct for every species or every tissue. In general, it seems that nerve tissue requires a less concentration of an anesthetic for anesthesia than other tissues. But it is hardly justifiable to assume that this affect of anesthetics on permeability is peculiar to egg tissue. Pike embryos were found to behave the same as eggs up to the time of the development of kidney function, when the excretion of salts interferes with the method used to measure permeability. It is probable that anesthetics retard the increase in permeability of any cell by any "stimulus."

DISCUSSION

In 1910 I observed that chloroform, when added to the sea water, reduces the electric conductivity of sea urchin eggs. This experiment was not repeated, owing to lack of time and the large number of sources of error that must be guarded against in order to be sure that decreased conductivity indicates decreased permeability. Osterhout,[10] by finding a tougher material, was able to show that anesthetics decrease the permeability, at least of certain plant cells. Joel[11] found that anesthetics decrease the permeability of erythrocytes. The question arises whether anesthetics prevent increase in permeability by decreasing permeability. If a cell is absolutely impermeable its permeability cannot be decreased. The Fundulus egg is so nearly impermeable to salts that it would be extremely difficult to measure a decrease in permeability. The pike egg may be more permeable to salts but it would be no easier to measure a decrease in permeability, owing to the delicate nature of the egg and danger of non-uniformity of material. It seems probable, however, that the anesthetic and toxic substances act on the same constituent of the cell surface or plasma membrane. If the cell surface is composed of a mosaic of different constituents,

[10] Osterhout: Sci., 1913, 111.
[11] Joel: Pflüger's Arch., 1914.

and one constituent is permeable, the cell is permeable. But if one constituent is impermeable the whole cell is not necessarily impermeable, since diffusion can take place through the other constituent. If the $NaNO_3$ makes the cell permeable by acting on a protein it is difficult to see how the anesthetic could antagonize this effect by acting on a lipoid.

Stimulation and anesthesia seem to be antagonistic states. I have shown that the permeability of striated muscle is increased on stimulation[12] and that the permeability of the eggs of the sea urchin and the frog increases when they pass from the state of repose into that of activity.[13] The question arises whether this is true of other cells, such as those of glands. In order to decide this we must discuss the psycho-galvanic reflex.

If a constant electric current is sent through the body from non-polarizable electrodes and the person is given a nervous shock, as by sticking a pin in him unexpectedly, the strength of the current is momentarily increased. Leva[14] showed that the degree of this change is in direct ratio with the number of sweat glands per unit area of the skin, and therefore concluded that the sweat glands produce this phenomenon. Gildemeister[15] was able, by improvements in the electric conductivity method, to show that this is due to the increased permeability, presumably of these glands. Apparently the gland cells are stimulated by the sympathetic nerves and their permeability is increased.

It seems, therefore, that the increased permeability on stimulation is a general phenomenon, and we might expect the action of anesthetics in preventing this increase to be general, also.

Warburg[16] has shown that anesthetics retard the oxidation of oxalic acid by blood charcoal to about the same degree as they decrease the respiration of nucleated erythrocytes. I have repeated and confirmed these experiments. Tashiro and Adams[17] have shown that the nerve fiber gives out less CO_2 when it is anesthetized. Although it was shown by Warburg[18] that the respiration of sea urchin eggs is only slightly

[12] McClendon: This Journal, 1912, xxix, 302.

[13] McClendon: This Journal, 1910, xxvii, 240, and ibid, in press.

[14] Leva: Münch. Med. Wochenschr., 1913, 2386.

[15] Gildemeister: Münch. Med. Wochenschr., 1913, 2289; see also Schwartz: Zentlbl. f. Physiol., 1913, xxvii, 734.

[16] Warburg: Pflüger's arch., 1914, clv, 147.

[17] Tashiro and Adams: Int. Zeitschr. f. Physik.-chem. Biol., 1914, i, 451.

[18] Warburg: Zeitschr. f. Physiol. Chem., 1910, lxvi, 306.

reduced during anesthesia, it seems generally true that anesthetics may antagonize oxidations by cells, oxidases and some inorganic katalyzers. The question whether permeability has any relation to the oxidative processes cannot be finally settled until we know more about the mechanism of the latter. At present we can, at most, make the generalization that in the presence of O_2 cell respiration varies more or less with cell permeability so long[19] as the cell is alive. The only observation that I know of that might appear to extend this rule to a dead cell is that of Warburg.[20] He found that the oxidation in the young erythrocytes of the goose is increased by freezing and thawing provided they are closely packed. Freezing and thawing causes hemolysis and increases permeability, and the hemolyzed cells might be considered dead.

SUMMARY

Anesthetics in the concentration that retards development (2–3 per cent alcohol or 0.5 per cent ether) tends to inhibit the permeability-increasing action of a $\frac{1}{10}$ molecular solution of $NaNO_3$ on the eggs and embryos of the pike (Esox).

[19] McClendon and Mitchell: Journ. Biol. Chem., 1912, x, 459.
[20] Warburg: Zeitschr. f. Physiol. Chem., 1911, 419.

NEW HYDROGEN ELECTRODES AND RAPID METHODS OF DETERMINING HYDROGEN ION CONCENTRATIONS

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The technique of hydrogen electrodes as applied to pure chemistry is in a high state of perfection[1] but the impression is sometimes given that these electrodes require a skilled physical chemist to use them correctly. It is true that some solutions give trouble, for example, a pure KCl solution. Starting with Merck's highest purity KCl, I recrystallized it five times in fused silica dishes and dissolved it in conductivity water, but could not obtain a neutral reading with the hydrogen electrode until I poured it boiling hot into the electrode and passed a rapid stream of hydrogen through it while cooling and while making the reading. This difficulty is never experienced with solutions containing considerable amounts of carbonates, phosphates or proteins, and hence biological fluids are less difficult than some inorganic solutions. In fact, it is not necessary with biological fluids to change the hydrogen in the electrode during the reading.

The time required to determine the reaction of a fluid depends chiefly on the time required to saturate the electrode with hydrogen. Since gold absorbs comparatively little hydrogen, it becomes quickly saturated. I found No. 36 gold wire very satisfactory from this standpoint but the electrodes made of it could not be so conveniently cleaned by heating, as platinum electrodes. Drucker made electrodes of films of iridium burned on Jena glass, but the same objection holds true of them. By reducing the thickness of the platinum, the saturation time may be reduced. I found that platinum foil 0.02 mm. in thickness, coated with platinum black, requires less than two minutes for saturation, provided it is separated from the hydrogen by only a film of the solution. Since the electrodes which Michaelis designed for rapid

[1] Ostwald-Luther: Physiko-chemischer Messungen, 3d ed. 1910, Leipzig.

work require thirty minutes for saturation[2] a considerable saving of time is thus accomplished by simply using narrow strips of thin foil instead of the wire that he used.

The chief difficulty in determining the H^+ concentration of biological fluids arises from the fact that they contain dissolved gases. The dilution of the hydrogen with other gases causes an error in the direction of greater acidity. The loss of CO_2 from the solution increases its alkalinity, hence the passage of CO_2 and O_2 from the solution into the hydrogen causes two errors which tend to oppose one another, and hence the reading might happen to be correct. Höber, Hasselbalch, Michaelis and others have guarded against the error due to escape of CO_2 from the solution. The method of Michaelis depends on the use of a very small volume of H_2 in ratio to the volume of the solution, and is best adapted to rapid work.

The chief difficulty with other gases is experienced in determinations on arterial blood. The oxyhemoglobin gives out so much O_2 into the H_2 as to cause a greater error than arises from the escape of CO_2. Milroy[3] centrifuged the blood and then poured it through the air into the electrode. In order to obviate errors arising from this procedure, as well as other errors, and to shorten the time required for a determination, I designed the following electrode:

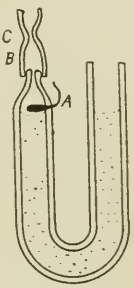

Fig. 1

This electrode consists essentially of a U-tube with one end constricted, figure 1. Next to the constriction, a strip of platinum foil, 0.02 mm. in thickness or thinner, is fused through the glass so that the plane of that portion of the foil which protrudes into the interior of the tube passes through the axis of the tube. The free end of the foil, *A*, is bent up so that when a loop of wire is passed over the constricted end of the tube electric contact will be made with the foil. A short piece of rubber tube, *B*, is attached to the constricted end of the glass tube, and closed with a small Langenbeck clip or pinch cock at *C*. The rubber tube is as short and of as small bore as practicable, and its free end is connected with a hypodermic needle. The large end of a hypodermic needle is filed down so that it may be inserted into the rubber tube. The platinum is coated with platinum black and cleaned in the usual manner.

[2] Michaelis: Die Wasserstoffionenkonzentration, 1914, Berlin.
[3] Milroy: Quart. Journ. Exper. Physiol., 1914, 141

Before filling the electrode, the needle is dried thoroughly be means of a suction pump and filled with oil. It may then be boiled for sterilization. The rubber tube is filled with a concentrated solution of hirudin in water or Ringer. The needle is inserted into the artery or vein and the Langênbeck clip removed. The U-tube is held in such a position that only the first few drops of blood come in contact with air, as this first blood covers and protects the rest of the blood. When the U-tube is filled, the Langenbeck clip is put on the rubber tube and the needle is removed.

The U-tube is now placed in the shield of a centrifuge, counterbalanced, and centrifuged a few minutes. It is then removed and a pipette inserted into the free end of the rubber tube. By sucking on the pipette and at the same time partially opening the Langenbeck clip, the blood corpuscles that remain in the rubber tube are removed. A tube from which pure hydrogen is flowing is instantly put in place of the pipette, without admitting any air. By cautiously opening the Langenbeck clip, hydrogen is admitted until the platinum foil is surrounded by the gas. A film of plasma adheres to the platinum and sides of the glass tube and establishes electrical connection with the blood below. After waiting two minutes for the platinum to be saturated with H_2, the U-tube is shaken so as to bring a fresh portion of the plasma in contact with the platinum, and the reading is immediately taken. Care should be taken not to shake so hard that any of the corpuscles rise high enough to liberate any oxygen into the hydrogen. In order that no time be lost in connecting this electrode with the calomel electrode, a ball of cotton cord soaked in a saturated solution of KCl is kept at hand and a piece cut off previously, with which to make the connection. The inclosure of this conducting cord in a tube is unnecessary.

Since the reading can be made in a few seconds with a proper potentiometer, the CO_2 does not have time to diffuse out of the film of plasma covering the platinum and cause an error. Since the erythrocytes have all been precipitated away from the surface layers, and the exposure is but little more than two minutes, the contamination of the hydrogen with other gases is very slight, and the error due to this unmeasurable.

This electrode has been indispensable in my work on blood, but it can be used with any biological fluid. In studying stomach or duodenal contents, the rubber tube may be connected to the smallest size stomach or duodenal tube, having a strainer (bucket) on the end that

is swallowed. The filling of the U-tube may be assisted by aspiration through a rubber tube attached to its free end, but the suction should not be sufficient to cause bubbles to appear in the fluid.

In case the readings are made at room temperature, it will be necessary to insert the U-tube in mercury in order to cool it with sufficient rapidity. If blood or any other fluid that requires to be centrifuged is used, the centrifuge shield may be filled with water so that most of the cooling takes place without loss of time.

If extreme accuracy is not required and the fluids to be investigated, contain no oxyhemoglobin, the electrode designed by Michaelis may be used, provided thin platinum foil is substituted, and a bulb is blown on the open end of the glass tube to prevent spilling while introducing the H_2. A strip of foil 0.05 mm. thick may be drawn through a small rubber stopper by means of a needle and thread, instead of sealing it in a glass stopper. This foil may be cleaned by heating, provided the stopper is first wet with distilled water, whereas a glass stopper is liable to crack.

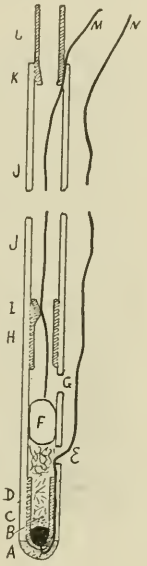

Fig. 2.

In measuring the acidity of the gastric contents, it was found possible to lower an electrode into the stomach. The apparatus designed for work on the stomach contents consists chiefly of a rubber tube 60 cm. long and 3 mm. bore, and two No. 40 silk covered copper wires, that were coated with rubber cement and dried several times (fig. 2). One wire, *M*, extends through the rubber tube, *JJ*, and the other, *N*, passes down outside of it until by entering the hole, *E*, it connects with a platinum wire that is fused into the lower end of a short piece of glass tube that is inserted into the rubber tube. The lower end of the glass tube and copper-platinum junction is covered with sealing wax, *A*. A drop of pure mercury is dropped into the lower end of the glass tube so as to connect with the platinum wire at the level of *B*. Above the mercury a little calomel washed with concentrated KCl solution, *C*, is placed, and the rest of the glass tube packed with moist KCl crystals, *D*, and the hole, *E*, stuffed with cotton soaked in KCl solution. This forms a calomel electrode, and is separated off from the remainder of the tube by a short piece of glass rod, *F*. Above *F* several holes are cut in the rubber tube at the level of *G*, and from this point a fine platinized platinum wire extends through the lumen of the tube and is held in place by fusion

to a bump on the inside of a short piece of glass tube at the level of I. This platinum wire then connects with the wire *M* and the junction is coated with rubber. The rubber tube is connected at *K* with a tube, *L*, leading from a hydrogen generator, and a slow stream of H_2 passes down the rubber tube and out at *G*, thus converting the platinum wire from *H* to *F* into a hydrogen electrode. Whereas the results are not quite as accurate as those obtained with larger electrodes, I think them sufficiently accurate for stomach contents, where there are such great individual variations. It is necessary to have a source of hydrogen of sufficient pressure to prevent the stomach contents from rising in the tube higher than *H*. Fresh crystals of KCl must be put in *D* before the apparatus is used, and the end of the tube from *A* to *G* may be immersed in saturated KCl solution so as to keep it moist until it is swallowed. A correction is made for hydrogen pressure (subtract 0.17 mv. for 1 cm. increase in pressure).

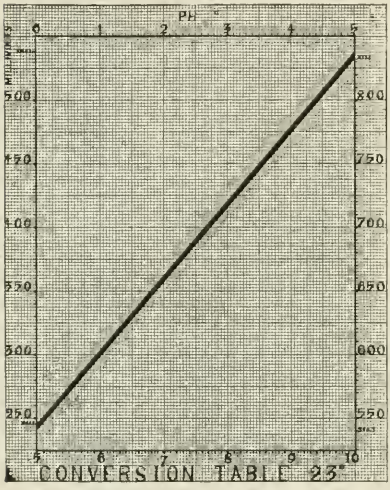

Fig. 3

The time necessary to calculate the H^+ concentration from the potentiometer reading may be saved either by using a conversion table or making a potentiometer that reads off directly the H^+ concentration. It is most convenient to express results in the form of the hydrogen ion exponent, PH. Thus 0.001 normal is 10^{-3}, or PH = 3. Figure 3 is a conversion table to be used at 23°, provided the hydrogen electrode is connected with a calomel electrode of the saturated type (containing KCl crystals). The coördinates are to be followed only to the edge of the diagonal band. Starting with the potentiometer reading in millivolts on the ordinate, the corresponding PH on the abscissa may be read.

The temperature coefficient is small and is practically zero for an H^+ concentration of 0.0001 normal (PH = 4). That is to say a 0.0001 normal solution of hydrogen ions will give a reading of 481 millivolts whether the temperature is 19°, 25° or 37°. If 23° is taken as the standard, at 19° there is a variation of +3 millivolts for PH = 0 and

-5 mv. for PH = 10. At 37° the variation is -11 mv. for PH = 0 and $+16$ mv. for PH = 10.

Since the PH of gastric juice is not far on the acid side of 4, that of urine is about 4 and that of the other biological fluids (except those containing much bile) is not far on the alkaline side of 4, a slight variation of the temperature from 23° does not make a serious error in using this conversion table or a potentiometer reading the PH directly.

None of the potentiometers on the market can be easily adapted to read the PH directly, but I made one that will serve all such purposes. It consists of 2200 cm. of No. 30 hardened German silver wire stretched over cross section paper. By means of a Weston cell, and 2 movable contacts, the current of a lead storage cell passing through a variable length of the wire is so adjusted that each centimeter has a fall of potential of one millivolt. By means of a conversion table the PH is marked on the cross section paper. We thus have a scale reading the PH instead of the millivolts. See also the description of a direct reading potentiometer in this journal.

SUMMARY

The method of H^+ concentration as used by Michaelis is modified so as to reduce the time necessary for a determination from forty minutes to about two or three minutes.

An electrode which eliminates errors due to O_2 (from oxyhemoglobin) and CO_2, is described. An electrode that may be lowered into the stomach is also described.

A potentiometer reading the H^+ concentration directly, instead of millivolts, is described.

A DIRECT READING POTENTIOMETER FOR MEASURING HYDROGEN ION CONCENTRATIONS

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One disadvantage of the use of the hydrogen electrode in determining the hydrogen ion concentration of biological fluids is the time required to calculate the results, and the liability to error by one not experienced in the use of mathematics. In order to avoid part of the calculation, some workers stop the calculation as soon as the logarithm is obtained. These logarithms cannot be used in compiling statistical data in the same way as decimal fractions, and hence the calculation that is saved at one time may have to be done at another. The direct reading potentiometer does the entire calculation for you in zero time.

The difficulty in making a direct reading potentiometer for hydrogen ion concentrations lies in the numerous temperature coefficients that would have to be considered in making an extremely accurate and universal apparatus. But I have shown in a recent paper[1] the temperature coefficient of the whole apparatus is negligible in modern heated rooms with wall thermoregulators. During the summer the temperature variation would be serious only for determinations on blood, where the differences observed are comparatively minute. The error due to temperature is certainly not greater than the same error in reading a burette. The instrument is correct at 23°.

The potentiometer is made to be used in connection with a calomel electrode filled with crystals of KCl, because this avoids the danger of error due to the change in concentration of the KCl solution by diffusion or evaporation. A Weston cell is used. If it does not give the theoretical voltage, it should be standardized and its voltage marked on it.

The potentiometer may be made in two sizes, a large size that is easily made accurate and a small size with evident advantages.

[1] McClendon: This Journal, 1915, xxxviii, 180.

The large form is essentially a resistance wire 23,000 mm. long, with movable contacts. Suppose the Weston cell is correct, that is to say, the voltage marked on it is 1.0185 at 23° the temperature of standardization of the potentiometer, if the current from it is passed through 10,185 mm. of the wire each centimeter will have a fall of potential of 1 millivolt. A storage cell current is sent through and balanced against the Weston cell current by changing the length of the wire. Since the lead storage cell gives a current from about 1.85 to 2.23 volts, the length of the wire should be capable of being changed a corresponding amount. By means of the half dial, *C*, and the sliding contact, *D*, figure 1, the

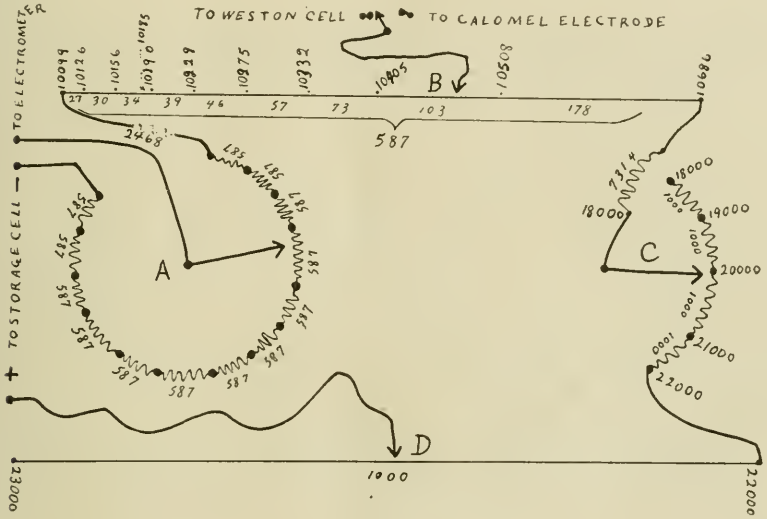

Fig. 1

length of the wire can be changed from 18,000 to 23,000 mm. corresponding to 1.8–2.3 volts. The slide wire, *D*, is 1 meter and hence out of proportion in the figure. In the figure, the length of wire between two buttons is marked by a number parallel to the wire and the total length of wire up to a button is marked perpendicular to the wire. The measurement of the wire for the half dial and slide wire, mentioned, need not be accurate, since the results are not affected by these measurements.

In order to determine the E. M. F. of the hydrogen + calomel electrodes, they are connected with the contacts on the dial, A, and the

slide wire, B. Since with a normal solution of hydrogen ions in the hydrogen electrode, the apparatus has a voltage of 0.2468, therefore 2468 mm. of wire are included between the dial and the slide wire. Since the moving of the decimal point in the hydrogen ion concentration one place, causes a change in voltage of 0.0587, there are 587 mm. of wire in the slide wire, B, and the same length between each pair of buttons on the dial, A. There are 14 buttons, and hence the length of the wire up to the slide wire is $(13\times587)+2468=10{,}099$, and hence 86 mm. on the slide wire is the place for leading off to the Weston cell, or a total of 10,185 mm. A temperature scale may be made for the Weston cell. It is 10,186 at 20°, 10,184, at 25°, and 10, 181 at 30°.

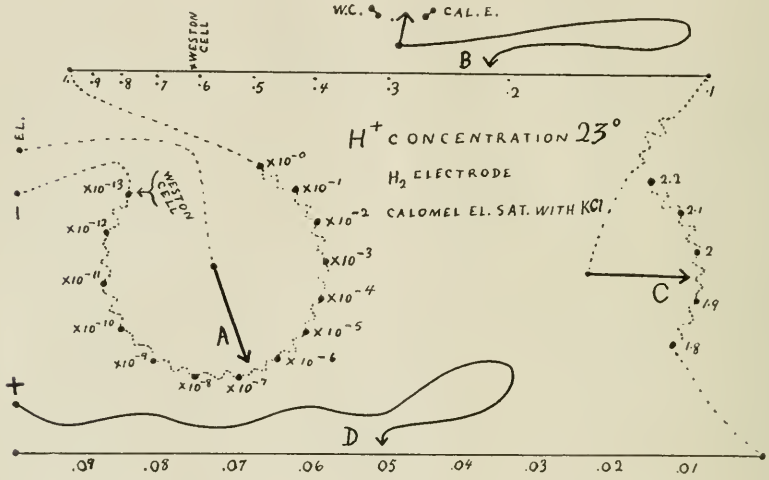

Fig. 2

The scale under the slide wire, B, for reading the hydrogen ion concentration, is logarithmic, and expressed in millimeters is as follows: $27+30+34+39+46+57+73+103+178=587$.

There is a binding post for the negative and one for the positive pole of the storage cell, one to the capillary electrometer, one for the Weston cell and one for the calomel electrode, and a double throw switch to connect the slide, B, either with the calomel electrode or the Weston cell.

A diagram of the face of the potentiometer is shown in figure 2. After the apparatus is set up in the usual manner, the contact of the dial, A, and the slide, B, and the double-throw switch are moved to the

points marked Weston cell, and the half dial, C, and slide, D, moved until a balance is obtained. Then the switch is thrown over and the dial, A, and slide, B, moved until a balance is obtained. The hydrogen ion concentration is then read off directly, for instance 0.4 on the slide wire and $x10^{-2}$ on the dial, or $0.4x10^{-2}=0.004$. Since it saves paper to write $0.3x10^{-12}$, for instance, than the equivalent decimal fraction, the reading is in the most convenient form for general work, without any calculation whatever. The range is from 10^{-0} to 10^{-14}, but this may be increased on the acid side as high as it is possible for the acidity of any solution to rise, in case it is desired to use the method on strong solutions of mineral acids.

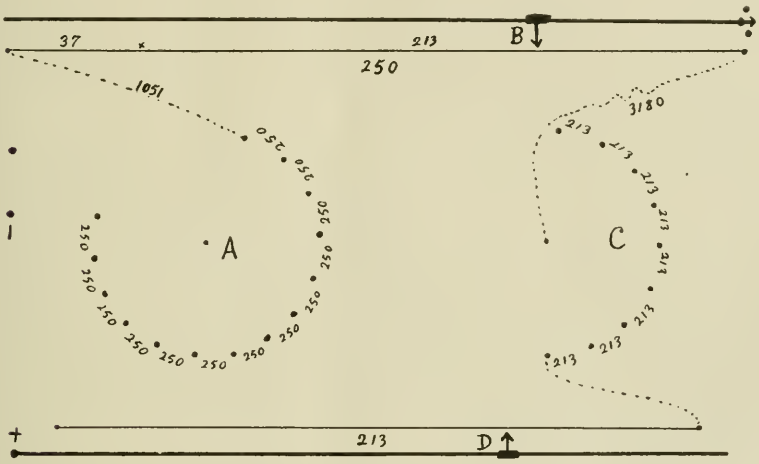

Fig. 3

The lengths of wire in millimeters for the small instrument are shown in figure 3. The length of the slide wire, B, is 250 mm. This is the same length as the logarithmic scale on small-sized slide rules, and logarithmic coördinate paper of this length may be obtained of Keuffel and Esser or other dealers. Therefore it is not worth while to calculate the divisions on the scale. Some samples of logarithmic paper are a little short, especially in a very dry room, but they may be moistened until they expand to the required length and then glued tight, especially at the ends, under the slide wire. By comparing figure 2, the orientation and labeling of the scale may be easily done. The mark on the slide wire scale for the Weston cell is 37 mm. from the left end. The

temperature correction for this point is so small that it may be judged with the eye. In general this mark should be shoved approximately 1 mm. to the right for 5° fall and 1 mm. to the left for 5° rise in temperature. At 30° it should be about 1.5 mm. to the left.

Instead of flexible wires to connect the sliding contact, *B*, with the double throw switch, and the sliding contact, *D*, with the binding posts, it is better to use brass rods with movable sleeves bearing the contact points, as in figure 3.

There are 250 mm. of wire between each two buttons on the dial, *A*, and 1051 mm. between this and the slide wire, *B*. If this wire is measured accurately the instrument will not need calibration for biological purposes, because the non-uniformity of well made wire, taken fresh off of the original spool, will be small, and notwithstanding slight errors in the wire the instrument will show differences accurately.

There are 9 buttons on the half dial, *C*, with 213 mm. of wire between each pair, and 215 mm. on the slide wire, *D*.

The more resistant the wire the better the storage cell will hold up. Manganin (86 Cu, 12 Mn, 2 Ni) invented by Weston, or Constantan (60 Cu, 40 Ni) of from 32 to 36 gauge, are the best materials for the wire, but German silver will do, especially if it is bare and strung in a ventilated box under the dials to avoid heating effects. Some of the wire should be saved to renew the slide wires when necessary. The connecting wires marked by heavy lines in figure 1, should be heavy copper (resistance free). The leads to the potentiometer do not have to be heavy, as only enough current passes through them to operate the electrometer.

I do not have a contact key on the potentiometer, because this key when released, must short circuit the electrometer, and a telegraph key connected directly with the electrometer, is used.

The small instrument is about 12x7 inches.

SUMMARY

The hydrogen ion concentration is read on the scale, *B*, figure 2, and the position of the decimal point read on the dial, *A*.

ACIDITY CURVES IN THE STOMACHS AND DUODENUMS OF ADULTS AND INFANTS, PLOTTED WITH THE AID OF IMPROVED METHODS OF MEASURING HYDROGEN ION CONCENTRATION

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The hydrogen ion concentration of the gastric juice and stomach contents after test meals is very well known.[1] According to Michaelis and Davidsohn[2] hyperacidity means a hydrogen ion concentration of from 0.011 to 0.088; average acidity means 0.028–0.0015 and hypoacidity means 0.00041–0.0000001.

Owing to the number of factors involved in the physiology of the stomach, as elucidated by the extensive work of Cannon and of Carlson, it occurred to the writer that the giving of a scant test meal, poor in protein, and the complete evacuation of the stomach all at once, might not reveal all that could be learned of the acidity of the stomach under normal conditions. Consequently an attempt was made to trace the progress of digestion after a normal meal, by lowering a hydrogen electrode into the stomach, and also by removing a few cubic centimeters every half hour by means of a very small tube with a strainer (bucket) on the end that is swallowed. The new departures in the technique are given in two other papers.[3]

It was found possible to keep the tube in the stomach all day except during the ingestion of the meals. After a little practice it was not very difficult to keep the strainer well in the interior of the main part of the stomach. The acidity at the cardia fluctuated more or less owing to the intermittent entrance of saliva. The pylorus became acid more rapidly than the rest of the stomach. But it is not true that the pylorus is always more acid than the fundus. My observations

[1] Fränkel: Zeitschr. f. Exper. Path. u. Therap., 1905, i, 431.
[2] Davidsohn: Zeitschr. f. Exper. Path. u. Therap., 1910, 398.
[3] McClendon: This Journal, 1915, xxxviii, 180 and 186.

indicate that the stomach contents begin to mix immediately after the ingestion of the food, and that the mixing is very thorough in about two to three hours.

The meals that were eaten were governed by the appetite, except in one case when a scant meal was purposely eaten for comparison. The real efficiency of the stomach to take care of these meals may be determined by finding the time required for the acidity of the main bulk of the food to rise to that degree most favorable to peptic digestion. This may be determined with little discomfort and little or no loss of nourishment.

The usual method of pumping out the entire stomach contents after an abnormal meal and titrating the acids and the salts of weak bases, is not calculated to shed very much light on the digestion. Such a procedure implies that acidity is some indestructible property of certain kinds of matter. The same procedure that is used to estimate matter, such as total nitrogen, cannot be used for studying acidity. In the first place new hydrogen ions are formed during the titration and the original number cannot be estimated in this way. The acidity may be determined more accurately by tasting than by titration. In the second place, the same number of hydrogen ions in the stomach may favor or retard digestion according to how they are distributed. Peptic digestion is most rapid in a hydrogen ion concentration of about 0.03 normal[4] and decreases when the acidity increases or decreases. The total stomach contents after being pumped out and mixed may be at the optimum acidity, whereas in the stomach, part may have been too acid and part not acid enough. It is much better to determine the hydrogen ion concentration of a sample taken from the large part of the stomach, away from the wall, because the main bulk of the food is in this location. Whereas digestion may commence immediately in the pylorus and next to the wall, the quantity of food so affected appears to be small, and general digestion is delayed until there has been a thorough mixing of the food with the gastric juice.

THE REACTION OF THE ADULT STOMACH AND DUODENUM

Figures 1 and 2 show a number of curves, representing the rise in acidity of the adult stomach during digestion. On the ordinates are the hydrogen ion concentrations and on the abscissae are the hours

[4] Sorensen: Biochem. Zeitschr., 1909, xxi, 131; Michaelis and Davidsohn: loc. cit.; Michaelis and Mendelssohn: Biochem. Zeitschr., 1914, lxiv,

after finishing the meals. Curves 1–4 are taken from the same individual to show the effect of meals of varying bulk, and hence varying protein content. Curve 1 was taken after a light meal of milk, curves 2 and 3 after average meals and curve 4 after a heavy dinner. Curve 5, from another individual, was taken after a meal that was not only light but also very poor in protein (chiefly carbohydrates). It is clearly seen that the heavier the meal and the more protein it contains, the more slowly the acidity rises. This is due to the fact that the protein neutralizes the acid and the acidity of the stomach cannot rise very high until all of the protein is changed into acid albuminate.

The curves in figure 2 are all taken from different individuals and in general steeper than those in figure 1. This is due to personal characteristics and not to the protein content. Whether this personal difference is due to difference in rate of acid secretion by the stomach, or difference in the quantity of saliva swallowed, is not clear. Curve 12, showing a very slow rise after a medium meal, was taken from a person having a very sensitive throat, and noticeably large quantities of saliva were swallowed. In the average individual, the tube produces no stimulation of the mucous membranes. Curve 11, taken after a light meal, rises steeply, whereas curve 6 taken from another individual, after a light meal, chiefly carbohydrate, does not rise so steeply.

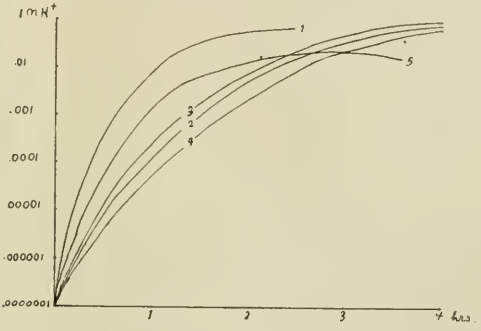

Fig. 1. Curves 1–4, male, 34 years; curve 1, luncheon, 500 cc. milk. Curve 2, breakfast, 1 orange, 250 cc. milk, 200 cc. water, 2 eggs, 2 slices of buttered toast. Curve 3, luncheon, roast lamb, potato, bread and butter, lettuce, ice cream. Curve 4, dinner, soup, beefsteak, potato, bread and butter, tomato, 250 cc. milk, 200 cc. water, slice of pie. Curve 5, male, 32 years, breakfast, bacon, cereal, bread and butter.

It is well known or generally assumed that the height to which the acidity rises is a personal characteristic. This is well illustrated in curves 1–4 (all from the same individual) all of which reach the same maximum. Curves 7 and 8 are from two brothers and show the same maximum. This suggests that the acidity of the stomach is influenced by heredity.

After reaching the characteristic maximum, the acidity of the stomach remains constant for considerable time, at least until the greater part of the food has left the stomach. After this it is difficult to take a sample from the interior, that is, away from the wall, and fluctuations that are sometimes observed may be due to this fact. After all of the food has left the stomach the acidity may fall, due to cessation or decrease in the secretion of acid and also to the swallowing of saliva that is about neutral in reaction and contains protein capable of neutralizing acid.

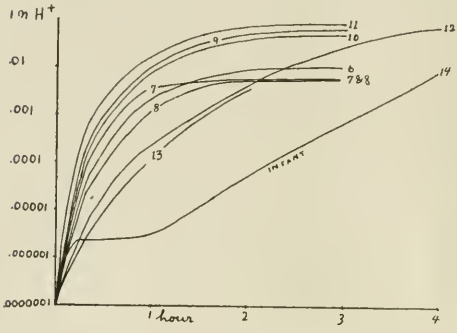

Fig. 2. Curve 6, male, 40 years, breakfast, toast and buttermilk. Curve 7, male, 24 years, dinner, ham, eggs, potato, pie. Curve 8, male, 19 years, dinner, veal roast, rhubarb, soup, buttermilk. Curve 9, male, 18 years, luncheon, 4 roast beef sandwiches, chocolate creams. Curve 10, male, 22 years, dinner, roast beef, 2 eggs, bread and butter, strawberries. Curve 11, male, 25 years, luncheon, ham, potato, bread and butter, coffee, sauce. Curve 12, male, 25 years, ham, potato, bread and butter, sweets, milk, rhubarb. Curve 13 (unfinished), female, 19 years, luncheon, vegetables, bread and butter. Curve 14, compiled from 27 samples of stomach contents of infants of the first month.

The hydrogen ion concentration of a number of duodenal samples taken with the duodenal tube were all very close to 2×10^{-8}. This may be generally true of all normal individuals, but it is not always true in disease. A sample which I determined for Dr. Schneider was very far from this value, but I will not give it here because he may wish to work up the subject from the pathological side.

THE CALIBRATION OF INDICATORS

During the experiments described, I took occasion to calibrate a number of indicator solutions and papers. Michaelis and Davidsohn[5] give a table of indicator solutions that are to be used after the Ewald breakfast. My results, after normal meals correspond closely with theirs except in case of methyl violet (and tropaeolin 00). Since my results correspond closely with those of Sorensen on non-protein solutions, this difference is not due to the higher protein content of my samples,

[5] Davidsohn: Zeitschr. Exp. Path. u. Therap., 1910, viii, 398.

and some of my samples had as low a protein content as those of Michaelis. The difference is probably due to the fact that Michaelis' samples were highly colored with tea. The yellow tea and blue indicator gave a green at 0.01 whereas in my experiments green never appeared, and in those of Sorensen it appeared at 1 normal and yellow appeared at 2 normal. I confirmed Sorensen's results on non-protein solutions and used different brands of methyl violet, Merck's 3B and Grubler's 5B, on gastric contents, but never obtained green at 0.01 when the contents were originally colorless. A similar difference was observed in the case of tropaeolin 00. In my tests it became orange at 0.01 and in those of Michaelis at 0.0033.

This trouble with the color of the sample increases as we go into the duodenum or test the urine. For this reason I calibrated a number of indicator papers to see if such a thing were possible. It is generally considered that indicator papers are less sensitive than the solutions. One reason for this is that the paper used contains substances that preserve the reaction (alkaline earths?). Bausch and Lomb congo paper begins to turn at 0.001 whereas some that I made begins to turn at 0.00005. I made a number of papers by soaking Schleicher and Schull's ash-free paper no. 589 in the indicator solutions and drying, and found them but little less sensitive than the solutions. The end of the paper is dipped into the sample and held there a little while. As the sample rises in the paper any coloring matter is adsorbed very quickly and prevented from rising further. The acid or alkali rises higher and gives the characteristic tinge to the indicator. Pure water rises higher, so that only the middle of the wet region should be observed. Proteins, and some other interfering substances also, are partially held back from ascending in the paper.

The original solutions and the color of the papers were as follows: methyl violet 0.2 per cent (violet), tropaeolin 00 2 per cent (deep yellow), dimethylamidoazobenzole 0.5 per cent (light yellow), Congo red, 0.5 per cent (red), methyl orange 2 per cent (yellow), alizarine sodium sulphonate 0.5 per cent (salmon pink), p-nitro phenol 2 per cent (light yellow), litmus 2 per cent (violet), neutral red 0.5 per cent (red).

The following table gives the observed changes in adult gastric contents: (−) = no change, B = blue, G = green, O = orange, R = red, V = violet, W = white and Y = yellow, (+) = a slight change:

Normal H^+.....	0.05	0.01	0.005	0.001	0.0001	0.00001	0.000001	0.000005
Met. violet.....	B	B	–	–	–	–	–	–
Tropaolin.....	R	O	–	–	–	–	–	–
2 met.a.a.ben....	R	R	O	–	–	–	–	–
Congo.....	B	B	B	V	V	–	–	–
Met. orange.....	R	R	R	R	O	–	–	–
Alizarine.....	Y	Y	Y	Y	Y	–	–	–
Litmus.....	R	R	R	R	R	+	–	–
P-nitro-ph.....	W	W	W	W	W	+	–	–
Neut. red.....	–	–	–	–	–	–	–	O

These indicator papers were tried on infants' stomach contents with the same result.

The indicator papers used on duodenal contents were: neutral red 0.5 per cent (red), rosolic acid 0.5 per cent (orange), cyanin 0.5 per cent (blue) phenol-phthalein 0.5 per cent (colorless). All of the normal duodenal contents investigated (0.00000005–0.00000001) affected the papers in the same way. Neutral red was changed to orange, rosolic acid was reddened, cyanin and phenol-phthalein were unchanged.

THE REACTION OF THE INFANT STOMACH AND DUODENAL CONTENTS

The work on the infant was done at the request of Prof. J. P. Sedgwick, head of pediatrics, University Hospital. The determinations of acidity were done by me with the hydrogen electrode, except for a few that were done by Dr. Rood Taylor under my direction. The samples were taken by Dr. Taylor, and the radiographs taken by Dr. F. S. Bissell, to both of whom my sincere thanks are due. Dr. Sedgwick gave me some references to the literature.

It was observed by Huenekens[6] that the acidity of the infant's stomach depends on the diet, and Hess,[7] that the titratable acidity of the stomach of the new born before it has taken any food, is high. I was able to obtain several samples of the latter and the acidity was 0.005, but the acidity of the empty infant's stomach is in general high, as will be shown later. Since the protein of the milk binds the acid, we should expect the acidity to be higher the less food is in the stomach, and such was found to be the case. In other words, the regulatory mechanism of gastric acidity in the infant is very imperfect.

Although 27 samples of the gastric contents of infants of the first month were investigated, I had no control over the manner in which

[6] Huenekens: Zeitschr. f. Kinderheilk., 1914, xi, 297.

[7] Hess: Amer. Journ. Diseases of Children, 1913, vi, 264.

the samples were taken, and an actual curve of the rise in acidity cannot be drawn. The entire stomach contents were pumped out and mixed in each sample. Since no relation between age of the infant and acidity was found, the various determinations may be used to construct a theoretical curve. Individual variations cannot be investigated, owing to the few determinations made on one infant. If we consider all samples taken one hour after nursing, for instance, it would be incorrect to use the mean acidity in computing the one-hour point in the curve, because a single stomach that emptied itself in one hour would have more influence than several stomachs that remained full for one hour. But if the variation curve of these determinations is made, the "mode" of the curve may be taken instead of the "mean." Most of the determinations are very near the "mode," and to use the "mode" has the same effect as throwing out the extreme variations. The results are as follows:

Time after nursing in hrs.	0.25	0.5	1.00000	1.5	1.75	2	2.5	3	4
H^+	0.000006	0.000005	0.000006	0.00006	0.00005	0.00012	0.00005	0.001	0.01

The acidity rises to 0.00012, two hours after nursing, and then drops to 0.00005, only to rise again very rapidly until the stomach is empty or until the next meal. This fall is due to the fact that there were not enough samples between two and three hours to obtain a good variation curve from which to determine the "mode." If the acidity curve is smoothed, we obtain the one shown in figure 2, curve 14.

Since woman's milk is neutral[8] and the earliest samples taken were of a much greater hydrogen ion concentration, it is probable that the stomach contains some gastric juice before the milk enters it. The acidity of this juice is high, as shown by curve 14. At the end of four hours there is practically no milk or even curds in the stomach, and at this time the acidity equals that of the adult stomach.

From a quarter to one hour after nursing the acidity remains practically stationary. During this time the protein is being transformed into acid albuminate and all of the acid secreted is used in the process. It was during this period and the hour following it that Davidsohn,[9] and others took their samples and observed the very low acidity. Schackwitz,[10] who did not take samples in the same way as Davidsohn, observed a greater acidity in a few cases.

[8] Davidsohn: Zeitschr. f. Kinderheilk, 1913, ix, 11.
[9] Davidsohn: Zeitschr. f. Kinderheilk, 1911, ii, 420.
[10] Schackwitz: Monatschr. f. Kinderheilk., 1903, xiii, 73.

It seems probable that the milk does not begin to leave the stomach until the end of one hour. From this time on, it is gradually diminished in quantity in the stomach and the acid that it secreted has less protein to neutralize it, and this protein is already partially saturated with acid, so that the acidity of the stomach continually rises. When the acidity has risen high enough for rapid peptic digestion, the quantity of milk left in the stomach is so small and passes out so quickly that it is safe to say that protein digestion practically does not occur in young infants' stomachs after a milk diet.

As a control on the collecting of the samples, the enzymes were determined. Pepsin was determined by the edestin method, trypsin by the casein method and lipase by the splitting of tributyrin as determined by the surface tension measured with Traube's stalagmometer (Michaelis). The stomach contained pepsin and gastric lipase (and milk lipase) but no trypsin.

Twenty-three samples of the duodenal contents of the same babies, taken by means of a catheter, were examined. Some of these were not bile stained but contained trypsin. By means of X-rays and determinations of bile and enzymes and the length of the tube swallowed, the origin of the samples was determined. There is no relation between the time after nursing and the acidity. I exclude three samples that were not bile stained, taken about four hours after nursing in the attempt to get duodenal contents, and showing an acidity of about 0.01. Dr. Taylor considered these as stomach contents, and I have included them in making the curve of stomach acidity.

The variation curve of duodenal acidity has a "mode" of about 0.0008, a maximum of 0.004 and a minimum of 0.00000015. When the stomach is very acid the acidity of the duodenum is lower than that of the stomach, but when the stomach is weakly acid the acidity of the duodenum is higher than that of the stomach. Very probably the acidity of the pylorus is always high, and causes the high acidity of some of the duodenal samples. The less acid samples have a greater admixture of bile. The bile is not sufficient to make the duodenal contents alkaline.

Unless the succus entericus is alkaline and abundant, the entire intestine must be acid. The question arises, what is the nature of the infant's digestion? The enzymes were determined in the same way was those of the stomach. I did not find it possible to distinguish between gastric and pancreatic lipase, but presumably gastric, pancreatic and milk lipase were present. Pepsin was present. Trypsin

was probably always present, although in three samples out of fifteen it seemed doubtful.

It seems certain that a high acidity is necessary for very rapid peptic digestion, but it is probable that after the pepsinogen is once activated by acid, slow digestion may take place at a very low acidity. Sorensen[11] observed peptic digestion in an acidity of abut 0.00008. According to Michaelis and Davidsohn[12] tryptic digestion ceases when the acidity is raised to 0.00001. We see therefore that the acidity of the infant's stomach is high enough to activate the pepsinogen, and that the reaction of the duodenum is as favorable for peptic digestion as it is for tryptic. Probably both forms of proteolysis proceed simultaneously in the infant's intestine. According to Rona and Arnheim[13] the reaction curve for erepsen is about the same as that of trypsin.

Very little is known of these enzymes, except the processes which they hasten and the conditions under which they act. Since it is certain that proteolysis occurs in the alimentary canal of the infant, the next thing to be determined is the reaction of the remainder of the intestine.

SUMMARY

The acidity of the adult stomach rises rapidly during the first 1.5 to 3 hours after a meal, after which it remains stationary until the food has nearly all left the stomach.

The rapidity of rise is less the heavier the meal and the more protein it contains, but it seems also to depend on the efficiency of the individual stomach.

The height to which the acidity rises is a personal characteristic.

The adult duodenal contents are slightly alkaline. The hydrogen ion concentration is about 0.00000002.

The acidity of the infant's stomach (of the first month) rises slowly during the time from fifteen minutes to one hour after nursing, after which it rises rapidly until the stomach is empty. Four hours after nursing it may be as acid as the adult stomach. The acidity is sufficient to activate the pepsinogen, but there is so little milk left in the stomach after the acidity has risen half way that the peptic digestion in the stomach seems unimportant.

The infant's duodenum is more acid than the average acidity of the stomach. Pepsin is always present and peptic digestion must take place.

[11] Sorensen: Biochem. Zeitschr. 1909, xxi, 294.

[12] Davidsohn: Biochem. Zeitschr., 1911, xxxvi, p. 280.

[13] Rona and Arnheim: Biochem. Zeitschr., 1913, lvii, 84.

THE PERFUSION OF THE MAMMALIAN MEDULLA: THE EFFECT OF CALCIUM AND OF POTASSIUM ON THE RESPIRATORY AND CARDIAC CENTERS

D. R. HOOKER

From the Physiological Laboratory of the Johns Hopkins University

Received for publication June 10, 1915

Using a perfusion apparatus the principle of which has already been described (1) the author has sought to perfuse the medullary centers in the dog. The method is now sufficiently advanced to report upon it and in this paper it is proposed to give the results obtained from a study of the effects of calcium and potassium upon the cardiac and respiratory centers.

An investigation of the effects of these salts upon the respiratory activity in the frog (2) while in part apparently conflicting showed clearly that, as compared with a balanced solution, predominance of calcium over potassium caused excitation and predominance of potassium over calcium caused depression. The same effects are now found in the case of the respiratory activity in the dog. It is found further that the salts in question have a definite action upon the tonicity of the cardiac centers, and it is interesting to note that while the resultant effect of potassium is to cause a slowing of both respiration and heart rate the one effect is apparently due to inhibition of one medullary center (respiratory), while the other is due to the excitation of another center (cardio-inhibitory).

The most recent work on the perfusion of the mammalian medulla is that of Herlitzka (3) and of Winterstein (4). Herlitzka fed defibrinated blood to the heart in dogs in which the circulation was limited to the head region. Under these conditions he noted the cardiac and respiratory movements and observed that the repeated head circulation of defibrinated blood, even when oxygenated was inadequate to preserve function for any length of time. The substitution of fresh defibrinated blood, however, temporarily improved conditions. Winterstein perfused new-born rabbits with saline solution at room temperature and observed that the addition of carbon dioxide and other acids produced

respiratory discharge in centers previously quiescent and concluded that the hydrogen-ion concentration of the perfusate governed the function of the respiratory center. Both of these authors review the earlier work in this field. Hirschfelder and Brown (4) have reported on certain pharmacological reactions in the central nervous system perfused with defibrinated blood but their results are not yet accessible to the writer.

Method. Such success as has been obtained by the present method is probably due in large part to the use of a new device for aerating the blood perfusate. The apparatus is shown in figure 1. It consists of an inverted bell-jar covered with a brass plate which clamps air-tight over a rubber washer. Openings in this plate permit the introduction of a thermometer, a tube for the gas mixture and a tube for the entering venous blood; a third tube leads outward through a trap for the escape of gas. In the center of the plate a hollow axle (*a*) carries a hub (*b*) which extends above and below the plate; below, it supports a flat rubber disc (*c*) of a diameter slightly less than that of the bell-jar, and above, a pulley (*d*) rotation of which causes the rubber disc to rotate. As the blood falls upon the rotating disc it is thrown against the side of the bell-jar and runs down in a thin film exposed to the contained air and collects in a suitable reservoir below. (The figure shows only the rubber stopper adapted to close the union

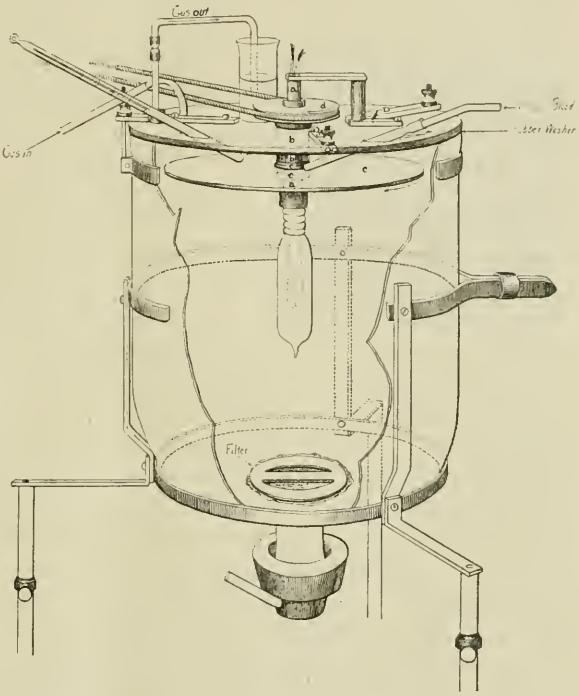

Fig. 1. Apparatus for aerating blood. Description in text.

between the chamber and the reservoir.) The hollow axle is closed by the entrance of insulated wires which lead to a small electric bulb by means of which the temperature is regulated. The bell-jar now in use is about 15 by 20 cm.

In the work here reported the perfusate consisted of washed corpuscles suspended in Ringer's solution. There was in consequence little tendency to froth. In numerous preliminary experiments, however, to test the maintenance of function in the medullary centers and in experiments directed to other ends defibrinated and hirudinized bloods have been used successfully. The apparatus permits the exposure of circulating blood to any chosen gas mixture and obtains adequate aeration without the frothing so likely to occur when protein solutions are agitated. So far as tried the size of the chamber is adequate to experimental needs. It is obvious that a larger one could be substituted if the volume of blood flow demanded it.

Technical procedure. The animal is prepared for perfusion under chloretone anaesthesia. The thorax is freely opened under artificial respiration after ligation of the internal mammary vessels. The right subclavian artery and vein are ligated. Loose ligatures are laid under the superior vena cava and left subclavian artery. The innominate is ligated close to the aortic arch and an inflow cannula is inserted distal to this ligature. An outflow cannula is placed in the right external jugular vein. The preparation is now connected with the perfusion apparatus and the ligatures about the left subclavian artery and superior vena cava are permanently tied. The head circulation is thus isolated except for anastomotic branches along the cord which doubtless unite the spinal branches of the intercostal with the spinal branches of the vertebral arteries. The arterial supply to the brain by the latter paths is insufficient to maintain life. A more serious difficulty lies in the venous anastomoses along the cord by which path there is a continuous seepage of blood from the head circulation into the body. The technical difficulties of occluding this path are so great that it has seemed best to ignore it at least until experimental results are likely to be invalidated by the entrance of chemical substances into the systemic circulation.

After the perfusion is established 300–400 cc. of blood are allowed to waste from the left external jugular vein before the latter is connected to return the perfusate to the apparatus. The isolated circulation as thus accomplished includes the neck parts and the whole head as sup-

plied by the branches of the carotid and the external carotid. The perfusate enters by way of both carotids and the right vertebral artery.

During an experiment artificial respiration is maintained to preserve the cardio-vascular system as a recorder of the medullary effects on this system. The heart-rate has been recorded with a Hürthle manometer connected with the femoral artery and the respiration by recording the movements of the epigastrium.

With the procedure as stated there is a possibility that spinal and vertebral vascular anastamoses might connect the systemic with the isolated circulation and that the former might in part contribute to sustain medullary life. Opposing this possibility are these facts: (a) medullary life ceases at once after ligation of both carotids and both vertebrals or upon stopping the perfusion in an experiment. It is interesting to note, however, that when the medulla has been isolated and perfused for some time the respiratory center will continue to discharge for a little while after the perfusion has been stopped just as the isolated mammalian heart will continue to beat under like conditions. (b) Medullary activity (respiratory movements) continues on the isolated circulation long after the heart has stopped beating. In some experiments lasting an hour or more the heart was not beating at any time.

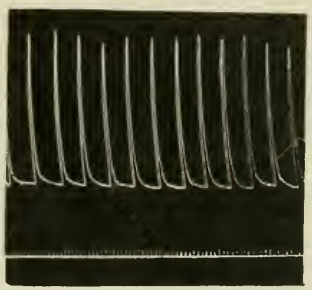

Fig. 2. Experiment of February 10. Tracing of the respiratory movements from a dog in which the head was perfused with defibrinated blood. Record 2 hours, 15 minutes after perfusion was begun. Heart ceased beating 30 minutes and eyelid reflex disappeared 2 hours after perfusion was begun. Perfusion pressure 60 mm. Hg. Upstroke indicates inspiration. Time in seconds.

Figure 2 is reproduced to indicate the reliability of the method employed. It shows the respiratory movements in a dog two hours and fifteen minutes after perfusion was begun. The perfusate consisted of defibrinated dog's blood diluted with about an equal volume of Ringer's solution. The perfusion pressure at the time the record was taken was 60 mm. Hg. Respiratory movements continued regular for ten minutes longer or for a total period of two hours and twenty-five minutes. In this experiment the heart stopped beating in the first half hour, a fact which supports the assertion that the isolation of the medulla is complete.

It is further of interest to note that the eyelid reflex disappeared thirty minutes before the respiratory center ceased to discharge; in the majority of experiments, however, the eyelid reflex persisted longest. It should be stated that this is the longest period the respiratory center has been kept alive. Because the heart had stopped beating no experimental procedures were instituted; this undoubtedly contributed to the period of activity of the preparation because it has been repeatedly observed that changing the experimental conditions in preparations with such a narrow margin of safety markedly reduces the period of survival. Nevertheless a number of experiments were continued successfully for two hours or more. The temperature of the blood in the venous outflow cannula was about 32° C.

Experimental. In the present experiments dogs' corpuscles suspended in salt solution made up the perfusate. An anaesthetized animal was bled, transfused with Ringer's solution and bled again. The blood was defibrinated and the corpuscles were separated in a centrifuge. The corpuscles were then washed twice in and finally suspended in the following solutions:

per cent		*per cent*		*per cent*
NaCl..... 0.90	NaCl..... 0.90		NaCl..... 0.90	
$CaCl_2$..... 0.03	$CaCl_2$..... 0.06		KCl..... 0.06	
KCl..... 0.03				

The solutions, tested before the corpuscles were added, were all slightly alkaline. The Ringer's mixture had a value of $P_H = 8.4+$; the potassium solution $P_H = 8.2$; while the calcium solution fell between the others. This variation in H ion concentration, if it prevailed after the corpuscles were added, was in all likelihood too small to affect the results. Furthermore, it will be noted, the potassium solution which depresses respiratory activity tends to be more acid than the calcium solution which acts as a stimulant.

In order to obtain the requisite volume of perfusate the corpuscles were suspended in a volume of salt solution about twice as great as that of the serum. While this dilution was not determined accurately in the several experiments care was observed to have the dilution the same in the suspensions used in any one experiment. The perfusate as thus prepared was kept on ice over night, in some cases over two nights, before being used. It was hoped that the washing would yield a final solution free of calcium or potassium when the absence of one or the other of these salts was desired so that the results might be correlated

with those obtained from the frog referred to in the introduction of this paper. This was not possible, however, as the supernatant fluid was never free of either calcium or potassium as shown by analysis. The experiments therefore bear only upon the effect of a preponderating amount of calcium and of potassium. The perfusate was aerated in all the experiments with pure oxygen.

Figure 3 shows the stimulating effect of a predominance of calcium. Between the marks the perfusate low in potassium and high in calcium content was substituted for the balanced solution. The respiratory center, previously quiescent, is caused to discharge and the heart-rate is markedly increased. This record, in common with others, shows a latent period of considerable length. The construction of the perfusion system is such that the change of perfusion solution is made some distance from the organ under investigation which no doubt accounts in large part for the delayed effect since some time must elapse before the new solution reaches the medulla. After the return to the control perfusate the respiratory movements quiet down and the heart-rate, somewhat more slowly, returns to the former rate.

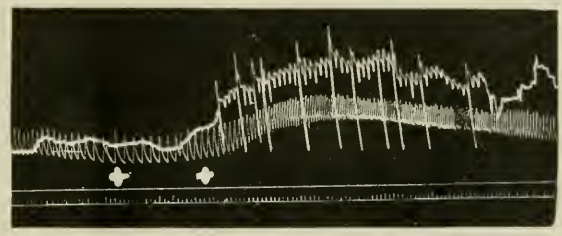

Fig. 3. Experiment of February 9. To show the effect of a preponderance of calcium on the heart-rate and respiratory movements. Downstroke indicates inspiration. Time in seconds.

Figure 4 shows the depressant effect of a predominance of potassium. Between the marks the perfusate low in calcium and high in potassium content was substituted for the balanced solution. A transitory increase in respiratory activity is noted which is followed by a period of complete depression. This momentary increase of respiratory activity was observed in several but not in all the records. The analagous condition of momentary depression when changing to the calcium-strong perfusate was also occasionally seen. This effect is not unlike that sometimes exhibited by the isolated heart when the feeding solutions are changed. Along with the depression of respiratory activity there is a marked slowing of the heart-rate. This slowing is taken to be a vagus effect caused by stimulation of the cardio-inhibitory center since it largely disappears after vagus section. The conclusion therefore

seems inevitable that potassium inhibits one center in the medulla while stimulating another. The data from this series of experiments, in which the cardiac nerves were intact, are presented in Table I.

An analysis of the response of the cardio-inhibitory and cardio-accelerator centers independently of one another was attempted after section of the vagus or accelerator nerves. These results are collected in Table II.

In the single experiment in which the accelerator nerves were cut potassium increase produced a retardation of the heart-rate comparable to that observed in the animals with cardiac nerves intact. The in-

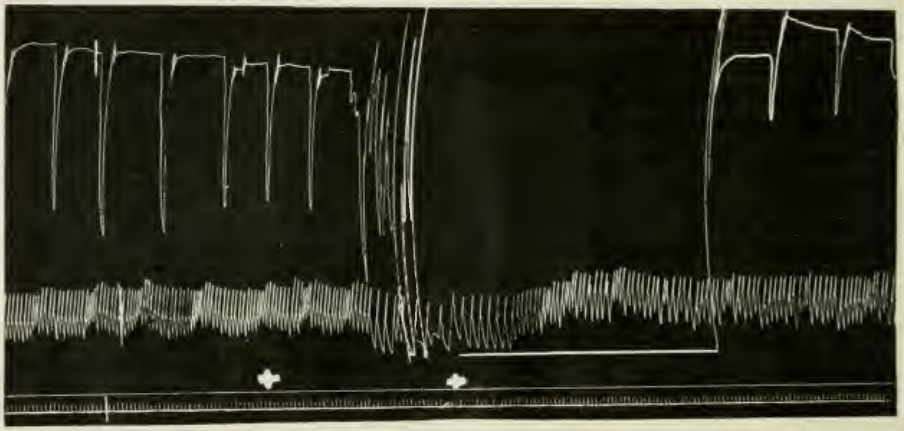

Fig. 4. Experiment of February 2. To show the effect of a preponderance of potassium on the heart-rate and respiratory movements. Downstroke indicates inspiration. Time in seconds.

crease in heart-rate following calcium increase was relatively insignificant. The result is, however, decisive enough to justify the belief that calcium exerts some inhibitory effect on the cardio-inhibitory center.

Five experiments were directed to show that calcium increase stimulates the cardio-accelerator center directly. The difficulty in this procedure is to obtain a slow heart-rate after vagus section so that accelerator effects will be apparent (6). In four of the experiments a slowing of the rate was attempted by reducing the pulmonary ventilation (7); the result in one of these was negative, in two it was insignificant and in the fourth the increase amounted to twenty beats per minute. In the last of these there was a very definite increase in the amplitude of

beat. In the fifth of this series of experiments the heart-rate was slowed by pilocarpine in the systemic circulation. By this procedure (experiment of June 4) a very definite increase in both rate and amplitude of beat was obtained by calcium increase.

TABLE I

Respiratory and heart-rates per minute before and during medullary perfusion of solutions containing a preponderance of calcium or of potassium. Cardiac nerves intact. The records were counted for 30 seconds.

	RESPIRATION		HEART RATE		RESPIRATION		HEART RATE	
	Before Ca	During Ca	Before Ca	During Ca	Before K	During K	Before K	During K
Feb. 2....					3	0	72	30
					18	0	104	30
Feb. 9....	0	52	38	122				
	0	12	34	124				
Mar. 5....					18	0		
					22	0		
					15	0		
Mar. 6....	6	12	70	82	6	0	54	28

TABLE II

Heart-rate per minute before and during medullary perfusion of solutions containing a preponderance of calcium or of potassium after accelerator section and after vagus section. The records were counted for 30 seconds.

	HEART RATE		HEART RATE		PROCEDURE
	Before Ca	During Ca	Before K	During K	
April 7.....	142	150	150	50	After accelerator section
May 1.....	124	134	116	102	After vagus section
May 21.....	102	110			After vagus section
May 22.....	144	164			After vagus section
June 4.....	11	20			After vagus section
	9	24			After vagus section

It seems therefore probable that both cardiac centers are responsive to each of the salts investigated. The effect of calcium is however much more pronounced on the accelerator center while the effect of potassium is much more pronounced on the inhibitory center. The

last described results in conjunction with several negative experiments emphasize further that the marked effect of the salts of calcium and potassium on the heart-rate seen in the experiments in which the cardiac nerves were intact is not due to a direct cardiac effect produced by the entrance of the perfused solution into the systemic circulation otherwise the heart would be influenced as much in the one set of experiments as in the other.

CONCLUSIONS

1. The medulla of the dog may be successfully perfused and the cardiac and respiratory centers will continue to function for a period of two hours or more on a saline solution in which dog's red blood corpuscles are suspended.

2. An apparatus is described which appears adequate for the aeration of blood used in perfusion experiments.

3. The effect of calcium predominating over potassium in a solution containing both of these salts is to stimulate the respiratory center and to increase the heart-rate. Conversely potassium predominating over calcium inhibits the respiratory center and slows the heart-rate.

4. When the accelerator nerves are cut potassium increase causes pronounced cardiac slowing while calcium increase causes slight cardiac acceleration. When the vagi are cut calcium increase causes definite cardiac acceleration and augmentation while potassium increase causes slight cardiac slowing.

5. The results obtained cannot be ascribed to incomplete isolation of the medulla.

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AN INTERPRETATION OF THE MEMBRANE MANOMETER CURVES AS AFFECTED BY VARIATIONS IN BLOOD PRESSURE

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Introduction. While there is a large literature on the subject of the tracings formed by the membrane blood pressure manometer, this does not bear directly on the changes in the membrane manometer curve as related to the different mechanisms by which the blood pressure changes were caused. During the past few years a large number of tracings have collected in the laboratories[1] in which the membrane manometer of the Harvard type has been used. As these tracings contain a large number of curves showing various types of circulatory phenomena, such as vasoconstriction and dilation, hemorrhage, etc., they have been studied with the idea of determining if there were not a fairly definite relationship of this kind; to learn, for example, if such phenomena as vasoconstriction, cardiac stimulation or depression, etc., are not represented by characteristic peculiarities in the manometer curves. It seemed probable that such an interpretation would disclose type curves that would give an indication of the cause of a change in blood pressure, which might be of especial value when other, possibly more exact, data were lacking. This we think has been accomplished.

No reference will be made to the form of the individual pulse record for the paper will be limited largely to a presentation of the modifications in the systolic and diastolic pressures caused by various physiological and pharmacological actions on the circulation. The pulse pressure will also be discussed.

Methods. The tracings were made with a Harvard membrane manometer attached by a Y tube to the carotid artery; to the other end of the tube a mercury manometer was attached, damped to give the mean blood pressure only and to prevent any changes in the membrane manometer that might arise from oscillations of the mercury. The dog

[1] With but few exceptions in the Pharmacology Laboratory at Cleveland.

was the usual experimental animal but occasionally the cat was employed. As the work extended over a period of several years, a number of rubber membranes of different thicknesses were used. This fact precludes an exact study of the relation between the amount or extent of the changes in the systolic and diastolic pressures and the degree of the rise or fall in blood pressure. Another fact that must be borne in mind is that the higher the blood pressure, the greater the tension on the membrane, so that a given impulse would probably cause a lesser change at a high level of pressure than at a low level. Neither of these factors, however, would alter the *type* of the curve, which is the question under discussion.

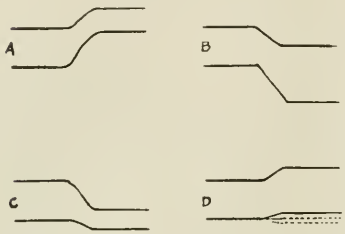

Fig. 1. Type curves formed by the membrane manometer. Upper level, the systolic pressure; lower level, the diastolic pressure. Type A—formed by general vasoconstriction and by increased heart rate. Type B—formed by general vasodilation and by decreased heart rate. Type C—formed by cardiac depression and by decreased volume of blood. Type D—formed by cardiac stimulation and by increased volume of blood.

Experiments showing fling of the lever are disregarded except when this followed the experimental procedure; note will be made of such results.

Method of presenting the results: The results will be presented according to the form of the curve displayed by the tracing rather than by the different physiological or pharmacological procedures. This classification seems to be somewhat simpler as most of the curves fall into definite group types. In all curves the upper limit represents the systolic and the lower limit the diastolic pressures.

The following type curves include the great majority of the curves:

Type A—In this type both systolic and diastolic pressures are elevated, but the diastolic much more than the systolic pressure, so that the amplitude of the pulse excursion is lessened (fig. 1a).

Type B—Both systolic and diastolic pressures are lowered, but the diastolic more than the systolic pressure, so that the amplitude of excursion is increased (fig. 1b).

Type C—Both pressures are lowered, but the systolic more than the diastolic pressure, so that the amplitude is lessened (fig. 1c).

Type D—The systolic pressure is raised somewhat while the diastolic remains the same or is slightly lowered or raised; in any case the amplitude is usually somewhat increased (fig. 1d).

To discuss the individual curves: Type A curve is formed from two distinct physiological phenomena, viz., vasoconstriction and increased heart rate.

Vasoconstriction. This was produced by several procedures, by faradic stimulation of sensory nerves (sciatic), by asphyxia and by large doses of nicotine and aconite, all of which stimulate the vasomoter center; also by the administration of epinephrin, pituitary extract, ergotoxin and ergot whose primary action is a direct stimulation of the arterioles although cardiac stimulation plays a part in the action. A great majority of the curves from these procedure fall under type A, that is as the blood pressure rises both systolic and diastolic pressures are raised but the elevation of the diastolic pressure is disproportionately great, so that the amplitude of the pulse excursion (pulse pressure) is lessened.

Fig. 2. To illustrate the early stage in the vasoconstriction type of curve. Experiment 33, dog; the upper curve is from the membrane manometer; the middle curve the mean blood-pressure from the damped mercury manometer; the signal line is the zero blood-pressure. 4—Sciatic stimulation; note the moderate rise in pressure, with the diastolic level raised considerably but the systolic very slightly.

The purest example of this class of vasoconstriction is that of faradic stimulation of the sciatic nerve (fig. 3 A), for the cardiac action of the drugs employed complicates their action somewhat, although as their predominant action is on the vessels the results are quite similar to that of pure vasoconstriction. The diastolic pressure is the determining feature of this type of curve, with the systolic pressure playing a secondary part. This point is well illustrated in experiment 33, figure 2. Four separate stimulations of the sciatic nerve caused a moderate rise in blood pressure each time; in each case the diastolic pressure was raised considerably while the systolic pressure remained the same or was very slightly elevated. The experiments were made with normal blood pressure and when the

pressure had been lowered by phenol or curare. A further illustration of the greater importance of the diastolic over the systolic pressure in this type of curve (vasoconstriction) is that as the blood pressure rises on stimulation of the sciatic nerve, the earliest effect to be observed in the curve is the prompt elevation in the diastolic level, usually before any change is noticeable in the systolic level.

Huerthle[1] found that, during the rise in blood pressure from sciatic stimulation in the rabbit, the pulse pressure was diminished. He, however, makes no reference to the importance of the diastolic pressure under such conditions.

Experimental data under type A curve. Faradization of the sciatic nerves: Forty-five uncomplicated experiments were made on 17 dogs; 35 curves were of type A and 9 of type D (increase in amplitude by a disproportionate elevation of the systolic level). The explanation of these exceptional cases is not clear; however the respiratory improvement and, secondarily, the cardiac improvement by the relief of a partial asphyxia may account for the increased systolic excursion. This does not include a large number of curves (19) made under abnormal conditions but all of which followed type A; they will be mentioned later. The experiments were made at all levels of blood pressure from 50 to 160 mm. and in all experiments the pressure rose considerably (from 10 to 50 mm.). While in general it may be stated that the greater the rise in blood pressure, the greater was the elevation of the diastolic level, yet the change in amplitude (pulse pressure) seemed to be fairly independent of the rise in pressure, not only in different but in the same experiments. Probably carefully conducted experiments with this end in view would show a closer relationship between the rise in pressure and the variation in the pulse pressure, but it must be borne in mind, as was mentioned in the introduction, that the higher the pressure the greater the tension on the manometer membrane, and this would tend to lessen the systolic excursion while not affecting the diastolic relaxation; this is a factor that would be difficult to estimate. It seems, then, that the form of the curve is the determining feature of this type of curve and not the extent of the change in the amplitude of the excursion.

Sciatic stimulation during low blood pressure from other procedures. The curve did not differ from that of the normal pressure. This type of curve was met twelve times while the pressure was low from curare, four times from phenol and three times from nitrite. With both curare and phenol there were experiments in which sciatic stimulation caused a rise in mean blood pressure with elevation of the diastolic pressure but without change in the systolic pressure as measured by the membrane manometer.

Asphyxia. Slight asphyxia was induced three times in one animal; each time as the blood pressure rose somewhat the manometer curve assumed the form of type A curve.

Aconite and Nicotine. With the great stimulation of the vasometer center from these drugs, type A curve was met once from aconite and twice from nicotine. In two other experiments with nicotine the pulse excursions were in-

[1] Quoted from Erlanger and Hooker (1).

creased somewhat by the greater elevation of the systolic level. As nicotine stimulates the heart as well as the vasomotor apparatus, this may explain the increase in systolic excursion. In a single instance nicotine paralyzed the vasomotor center; the blood pressure curve assumed type B, typical of vasomotor depression which is to be described later.

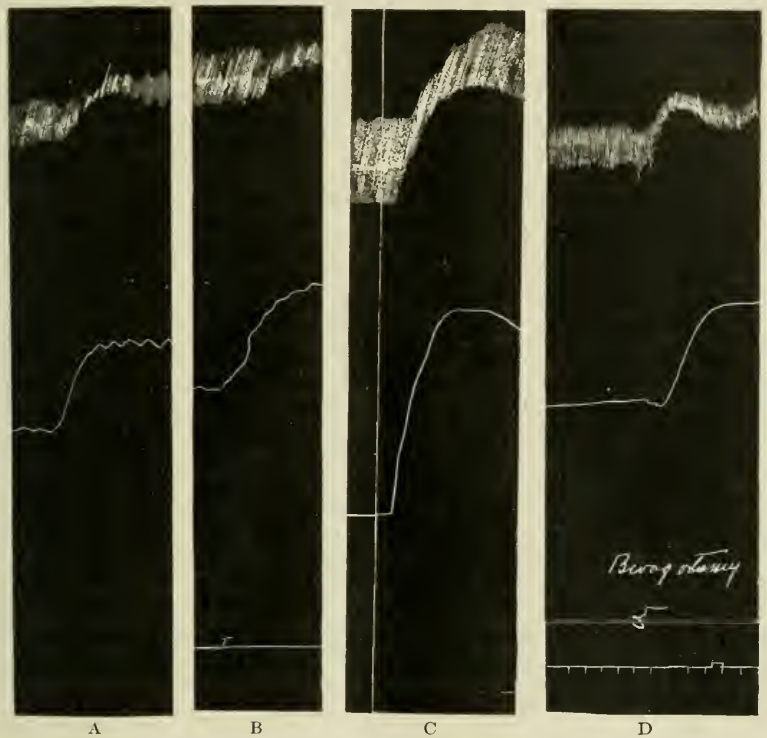

Fig. 3. Type A Curve—To illustrate vasoconstriction and increased heart rate. Upper curve is from the membrane manometer; middle curve is the mean blood-pressure from the damped Hg manometer. A—Sciatic stimulation; experiment 28. B—Injection of pituitary extract immediately after curve A. C—Injection of epinephrin; experiment 34. D—Section of the vagi; experiment 79. A, B and C illustrate the rise in pressure from vasoconstriction; D from section of the vagi.

Epinephrin. The curve of epinephrin resembles that of sciatic stimulation very closely in the majority of experiments (7 of 10). (Fig. 3 C) As the blood pressure rises the diastolic pressure usually is elevated before there is any change in the systolic pressure, and the maximum decrease in the excursion occurs, as a rule, during the rise in pressure; at the maximum level the amplitude of excursion becomes larger but still remains below the normal. This increase in the

excursion at the height of the curve may be due to the cardiac stimulation, for three times the amplitude was above the normal at this point but early in the rise in pressure there was a brief period in which the amplitude was lessened solely by the elevation of the diastolic pressure; these experiments are the three exceptional ones mentioned above so that in reality all the epinephrin curves follow type A. In these exceptional experiments, however, sciatic stimulation also caused an increased pulse pressure. When epinephrin is given in acute cardiac failure, of course the rise in pressure is accompanied by a greater increase in systolic than in diastolic pressure.

Pituitary extract. Type A curve was formed in five of seven experiments; in the other two both pressures were equally elevated; possibly the cardiac element was more marked in these cases. The curve at times resembles that of sciatic stimulation very closely as is shown in figure 3B, in which sciatic stimulation immediately preceded the injection of the pituitary extract. There may be considerable decrease in the excursion solely by the elevation of the diastolic level, with the systolic level remaining the same: in experiment 227, on the injection of pituitary extract the blood pressure rose from 75 to 130 mm. and the excursion was lessened from 24 to 10 mm. solely by the elevation of the diastolic pressure; however, too much stress should not be placed on but one experiment for there may have been a certain amount of fling of the lever that was not noted during the experiment and this would have given a greater original systolic excursion than really existed; the point that is to be emphasized is that the diastolic pressure is the determining feature of the pituitary curve.

Ergotoxin and ergot. Each drug formed type A curve in but a single experiment.

Type A curve from increased heart rate. The general form of the type is maintained in this group but as a rule the blood pressure rose more gradually than in the members of the previous group (especially sciatic stimulation and epinephrin) so that the changes were not so pronounced, although in a few instances there was little difference between the two classes (fig. 3 D). The heart rate was increased by section of the vagi or by the administration of atropin to paralyze the vagal endings.

Vagus section. In nine instances this resulted in an increased heart rate and a rise in blood pressure (average 35 mm.). All manometer curves assumed type A with moderate decrease in the amplitude of excursion, largely by the elevation of the diastolic pressure; occasionally the systolic level was but very slightly changed, if at all.

Atropin. When atropin caused an increased heart rate and a rise in blood pressure, type A form of curve was found in three of four experiments; in the fourth case type D was formed.

Erlanger and Hooker (1) have pointed out that acceleration of the heart, such as occurs after section of the vagi; as a rule diminished the pulse pressure, a statement that these results confirm.

Type B curve. In this type the amplitude of the excursions is increased by a great lowering of the diastolic pressure; the systolic pressure is also usually lowered but exceptionally may remain unaffected. The type is met during the fall in blood pressure from two distinct phenomena, vasodilation and cardiac slowing, conditions that are just the reverse of those that produced the preceding type of curve.

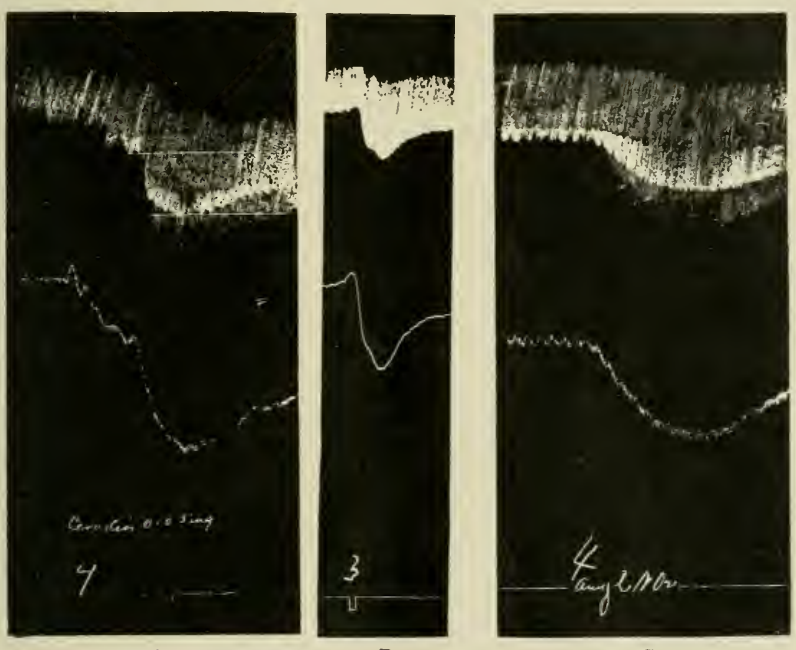

Fig. 4. Type B Curve—To illustrate the effect of the fall in blood-pressure from cardiac slowing and vasodilation. Upper curve is that of membrane manometer; the middle curve is the mean blood-pressure from a damped Hg manometer; the signal line is the zero blood-pressure. A—Cardiac slowing from cevadin; experiment 49. B—Vasodilation from nitroglycerin; experiment 90. C—Vasodilation from amyl nitrite; experiment 55.

The vasodilator group. The vasodilation was induced most typically and in the purest form by the administration of nitrites—amyl and sodium nitrites and nitroglycerin (fig. 4 B, C); also by chloroform, phenol, atropin (in large doses only), and by the intravenous administration of curare which probably causes peripheral vasodilation. The typical dilator action of chloroform and phenol, however, is modified

by their cardiac action. In this group the heart rate was practically unaffected.

The Nitrites. In all the experiments (19) with the nitrites as the blood pressure fell the diastolic level was greatly lowered, while the systolic level, though usually also lowered considerably, was frequently unaffected or but very slightly lowered; this caused a great increase in the amplitude of the excursion of the pulse-pressure. Too much emphasis should not be placed on the occasionally unchanged systolic level as the sudden emptying of the heart into the dilated vessels would undoubtedly tend to cause fling of the manometer lever. It is rather striking that there seemed to be a tendency for the systolic level to recover to the normal somewhat earlier than the diastolic level, but as many of the experiments were interrupted by other procedures, the evidence for this point is not complete.

There was no material difference in the curves of the three members of the group, amyl and sodium nitrites and nitroglycerin. There were twelve experiments with nitroglycerin (0.5 to 1.0 mgm. per kilogram), five with amyl nitrite and two with sodium nitrite (5 and 10 mgm). In each case there was a pronounced fall in blood pressure, varying from 25 to 65 mm; the experiments were made at all levels of pressure from 70 to 205 mm, without showing any striking differences in the manometer curves. The amplitude was more than doubled in several curves.

Chloroform. This has a twofold action, depressing both the heart and vasomotor systems. When administered not too rapidly by inhalation the vasomotor action predominates; when given by vein in suitable dosage direct cardiac depression can be obtained. The intravenous results will be discussed under the cardiac depressants.

Chloroform was given by inhalation seven times to six animals and in all of them type B curve was formed as the blood pressure fell. In a single experiment, in which the administration was pushed rather rapidly, the systolic pressure fell about as much as the diastolic, thus approaching the cardiac depression type of curve; the blood pressure also fell more rapidly in this experiment. The average fall in pressure was 50 mm., extremes of 20 and 90 mm.

Phenol. As the blood pressure fell from the intravenous administration of phenol in six of seven experiments type B curve was formed, thus indicating that the cause of the fall in pressure was vascular, at least in part, and for this there is direct evidence from the perfusion method elsewhere described (2). Direct cardiac depression is the other factor in the fall in pressure; there is, however, but a single instance in which there is evidence for this statement: in one experiment the systolic level fell more than the diastolic, thus decreasing the amplitude of the excursion in a manner that indicates cardiac depression (to be discussed later). In all experiments the blood pressure fell sharply, an average of 45 mm., extremes of 25 and 65 mm.

Atropin. When atropin caused a fall in blood pressure the curve assumed type B, indicating that the fall in pressure was due to vasomotor depression as the heart rate was not materially affected under the conditions of the experiment.

There were six experiments in which atropin in doses of from 0.1 to 1.0 mgm. per kilogram, and single experiments with 5 and 10 mgm., caused a mean fall in

blood pressure of 15 mm. (extremes 5 and 72 mm.). All but one tracing followed type B. The amplitude was increased a variable amount, usually moderately, but in one experiment from 5 to 35 mm., part of which was probably due to fling of the lever. The heart rate was somewhat increased twice and decreased once; in the other experiments there was practically no change in rate as the vagi were divided or previous doses of atropin had been given.

Curare. During the fall of blood-pressure from the intravenous administration of curare there is always a great lowering of diastolic pressure, and usually, but not always, the systolic pressure falls also; in either case there is a marked increase in the amplitude of the cardiac excursions. The lowering of the diastolic level precedes the fall in systolic pressure, and the former falls abruptly while the latter falls gradually, indicating that the lowering of the diastolic level is the essential cause of the fall in blood pressure. As this curve is of the same type as the nitrite curve it gives further evidence that peripheral vasomotor depression is the cause of the curare fall; peripheral because the vasomotor center is not depressed but rather stimulated (2).

Curare was given a total of 23 times to 23 dogs. The average fall in pressure was 40 mm. (extremes 12 and 95 mm.). In several experiments the systolic pressure was either raised slightly (five times) or remained unchanged (twice); with but one exception the blood pressure fall was considerably less than in the other experiments, so that when the curare action is marked the systolic level also falls. Fling of the lever may also account for the maintenance of the systolic pressure in such experiments. When the minimum blood pressure was reached, usually the systolic pressure had fallen considerably so that the pulse pressure was lessened, although there were numerous exceptions as noted.

Hales,[2] and later Bernard,[2] pointed out that a fall in blood pressure increases the amplitude of the pulse. Our observations confirm this statement as regards the fall in pressure from general vasodilation and from decreased heart rate; we wish to emphasize that the increase in the pulse pressure is brought about largely by the disproportionate lowering of the diastolic pressure. The lesser change in the systolic level is nicely put by Marey:[2] "If we assume that the more or less rapid and more or less abundant entrance of blood into the arterial system result from the excess of ventricular pressure over the pressure in the arterial system, then it is evident that a fall of arterial pressure would be exactly similar to an increase in the force of the heart beat, and there is, therefore, nothing surprising in this augmentation of the force of the pulse in all conditions that lower the blood pressure."

The effect on the pulse curve of lowered blood pressure from hemorrhage will be discussed later.

Type B from decreased heart rate. The curve is similar to that of the vasodilator group except that usually the systolic level is not lessened to as great an extent. The curve is simply that of the "vagus pulse" characterized by a prolonged diastole, during which the pressure falls greatly, while the systolic pressure is but moderately lowered or may

[2] Quoted from Erlanger and Hooker (1).

even remain unaffected; in any case the pulse pressure is greatly increased.

The heart rate was decreased by faradic stimulation of the peripheral end of the divided cervical vagus, by the intravenous administration of veratrum viride and its alkaloid cevadin, and in a single experiment by aconite.

Veratrum viride, as the tincture, was given in doses of from 1 to 10 mgm. per kilo of body weight; cevadin in 0.05 mgm. per kilo doses. In all there were 13 experiments on 11 animals and the two drugs gave similar results. With the administration of the drug the heart rate was markedly decreased so that the blood pressure fell from 30 to 70 mm. in each experiment. All experiments gave type B form of curve (fig. 4A).

Vagus stimulation gave results similar to veratrum in three cases. In other experiments the heart was completely inhibited so that the systolic pressure fell to zero.

Type curves C and D. In the preceding curves the characteristic feature has been the changes in the disatolic pressure; in the curves to be described next (C and D) the characteristic feature is the systolic variation in pressure although the diastolic pressure may also be considerably changed. In type C both levels are lowered, but the systolic much more than the diastolic level so that the pulse pressure is lessened. This type is met typically following sudden arterial hemorrhage and depends upon a decreased quantity of blood (fig. 5 B); and from cardiac depression. In type D the systolic pressure is somewhat elevated while the diastole remains unaffected or is slightly raised or lowered. The curve follows an increase in the volume of blood, either from the injection of defibrinated blood or normal saline solution into the bled animal, or from the injection of saline solution into the normal animal (fig. 5 A, C). Type D is also met from cardiac stimulation, although the experiments showing pure cardiac stimulation are somewhat limited in number. As the two curves follow one another experimentally it is simpler to discuss them together.

Hemorrhage. The animals were bled rapidly from the femoral artery in successive small hemorrhages until a very low point in blood pressure was reached; then either the defibrinated blood or normal saline solution, or each in succession, was injected into the femoral vein. At the onset of the hemorrhage the systolic level usually fell promptly, and the diastolic level also fell but to a much lesser extent, so that the amplitude of the pulse excursions was lessened. This effect was met with small hemorrhages of but 5 cc. per kilo and increased with succeeding hemor-

rhages, the pulse pressure becoming smaller and smaller as the total amount of blood withdrawn increased. The individual experiments showed considerable differences in the extent of the change in the maximum and minimum pressures because of the variation in the thickness of the membrane, so that it is not feasible to make quantitative com-

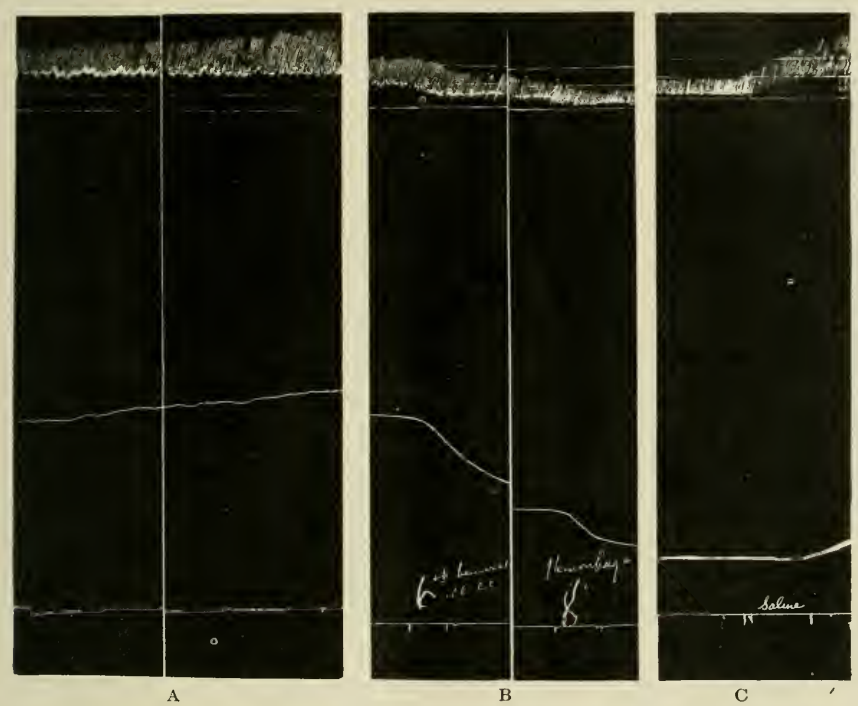

Fig. 5. Types C and D Curves—To illustrate the effect of saline infusion and hemorrhage; experiment 18, dog; the upper curve is from the membrane manometer; the middle curve the mean pressure from the damped Hg manometer. Curve A—Saline infusion before hemorrhage. Curve B—Hemorrhage, two portions of 10 cc. each. Curve C—Saline infusion after hemorrhage. Curves A and B have been interrupted.

parisons. Between the successive hemorrhages there was usually very little recovery in the systolic pressure but as a rule the diastolic pressure recovered somewhat.

Reinjection of blood and saline solutions. When the blood pressure had fallen to the minimum point compatible with safety, the defibrinated blood or saline solution were injected. As the pressure rose the ma-

nometer curves showed changes practically the reverse of those formed from the withdrawal of the blood, i.e., while both the systolic and diastolic pressures were raised, the systolic was raised more than the diastolic level so that the pulse excursions were increased. If the injections were sufficiently large, the curve returned to the normal.

The injection of saline solution into the normal animal. This resulted in a constant, though variable, elevation of the systolic pressure; the diastolic pressure was variable, slightly raised, unchanged or lowered a little. The amplitude of the excursions were increased by the predominance of the systolic rise. The slight lowering of the diastolic pressure is of little import as it may have been caused by fling of the lever.

Data on hemorrhage and saline infusion. There were 16 experiments on 12 dogs. The blood was withdrawn in 5 to 10 cc. portions until the minimum point in pressure was reached, about 30 to 40 mm. In 12 experiments the systolic pressure was lowered disproportionately so that type C curve was formed; in one experiment the maximum and minimum pressures were equally lowered and in three the curve was irregular but the diastolic pressure recovered before the systolic which is the usual occurrence. When the defibrinated blood (5 experiments) and saline solution (2 experiments) were injected there was a prompt elevation of the systolic level while the diastolic level was but slightly affected as mentioned above until rather large amounts were injected when conditions returned toward the normal.

Saline infusion into the normal animal. Ten experiments were made on six dogs, injecting from 5 to 35 cc. per kilo. All experiments gave an increased amplitude of excursion after type D curve. The diastolic pressure was slightly raised four times and slightly lowered or unaffected each three times. In each experiment the blood pressure rose moderately, average 13 mm., extremes of 4 and 20 mm.

Type C curve from cardiac depression. Direct cardiac depression was produced by the intravenous injection of chloroform or by saline solution saturated with chloroform. The drug, of course, depresses the vasomotor system as well, but by giving small doses by vein the primary action seems to be direct cardiac depression as the action is of such brief duration and recovery prompt and complete; in one instance, however, there was evidence of marked temporary depression of the vasomotor center without altering the cardiac depression form of curve. On the injection of 0.1 to 0.2 cc. of chloroform the blood pressure fell immediately and considerably, but returned to the normal usually within two to four minutes. During this period the cardiac excursions were greatly lessened, largely by a great lowering of the systolic level although the diastolic level was also lowered somewhat (fig. 6). Usually, also,

the diastolic pressure recovered before the systolic, which indicates weakening of the heart. The heart rate was not materially affected except in three instances when there was considerable slowing without altering the type of the curve. There were ten such experiments on six animals. The blood pressure fell an average of 45 mm., extremes of 20 and 67 mm.

Cardiac depression was also observed in ten experiments from *asphyxia* when the vasomotor center was paralyzed, thus preventing the usual rise in pressure from central vasomotor stimulation. In each instance as the pressure fell there was a gradual lowering of the systolic pressure before the diastolic pressure fell as well, thus following type C curve.

Experiments showing *cardiac stimulation* are suggestive only, for they are not free from the element of vasomotor action. Thus strophanthus gives the curve of vasoconstriction somewhat more frequently than what may be considered the type of cardiac stimulation, that is with the systolic level raised more than the diastolic level, thus increasing the excursion. With caffein the number of experiments with good membrane manometer curves is limited and these show a variable response: In two instances stimulation was indicated by a rise in blood pressure, the amplitude of excursion was either increased by a greater elevation of the systolic level or it remained unchanged with both pressures somewhat raised, both curves indicating cardiac stimulation. In other experiments, with fall in pressure, the systolic level was twice lowered more than the diastolic level, indicating cardiac depression; in still another instance vasodilation was indicated. In the acute fall in pressure from the intravenous injection the curve was also irregular, indicating either cardiac or vasomotor depression or both.

Fig 6. To illustrate cardiac depression. Experiment 91, dog; the upper curve is from the membrane manometer; the middle curve the mean pressure from the damped Hg manometer; signal line is the zero pressure. At the signal chloroform in saline solution was given intravenously.

The irregularity of the caffein curves is readily appreciated when it is recalled that the drug primarily stimulates the heart and depresses the vasomotor apparatus and later depresses the heart as well.

Discussion. After the experimental results had been classified as in the text, the explanation of the formation of the type curves was sought on *a priori* grounds and are here presented. The main factor in the formation of the curves is, of course, the physiological processes, modified, it may be to a certain extent, by the mechanical influences of the rubber membrane of the manometer. As the various types of phenomena were classified in the text as if they occurred in what may be called the pure state, that is, unmodified by other circulatory influences, the same plan will be followed in this discussion. It must be borne in mind, however, that the various factors that determine the circulation interact with, and modify, one another, so that probably a strictly "pure" type does not exist.

The vasoconstriction type. This is characterized by the disproportionate elevation of the diastolic level. The diastolic pressure is determined by the rate of blood-flow through the arterioles to the venous side of the circulation, therefore the greater the resistance offered by the arterioles during the vasoconstriction, the less will be the passage of blood through them and consequently the higher the diastolic pressure will rise. The systolic pressure is determined by the rate and force of the discharge of blood into the aorta; the greater the resistance in the aorta the smaller the quantity of blood that can be forced into it with a given strength of heart beat. The higher the diastolic pressure rises the nearer it approaches the systolic intracardiac pressure, and this means that with each systole a lesser quantity of blood will be discharged into the aorta because of the increasing resistance to the discharge, and the added increment of pressure will be less and less as the diastolic pressure rises. Therefore, as was stated in the text, it is the diastolic pressure that determines the vasoconstriction type of curve, with the systolic pressure playing a secondary part. The mechanical factor that might influence the form of the curve is that the higher the blood pressure the greater the resistance offered by the rubber dam of the manometer. This would tend to lessen the systolic excursion while not affecting the diastolic relaxation. However, as this type of curve is the same at all levels of blood pressure and with different manometer membranes, the mechanical factor is evidently of little importance in determining the type of curve.

The vasodilation type, characterized by excessive depression of the diastolic pressure is lowered by the rapid escape of blood through the dilated arterioles into the venous side of the circulation. The systolic pressure tends to approach the normal as the expulsive force of the heart remains

practically unchanged and because of the lessened resistance in the aorta to the systolic discharge. Thus the determining feature of the vasoconstriction curve is the diastolic pressure.

Cardiac depression. The systolic level is lowered by the lessened force of the cardiac contraction. The diastolic pressure is primarily unaffected as the resistance to the blood-flow from the arterial to the venous side is primarily unaffected.

Cardiac stimulation. The systolic level is raised because of the increased force of the systolic contraction. The diastolic pressure is not materially affected.

Change in the volume of blood. Decrease in the volume of blood would result in a lessened discharge, hence a lowered systolic pressure. Increase in the volume would result in a greater systolic output and a higher systolic pressure. In either case the diastolic pressure would be a secondary feature. This agrees with the experimental findings. The change in the systolic pressure was greater in the case of hemorrhage as the compensatory mechanism would not be as efficacious as when the volume of the blood is increased by the addition of saline solution. When the infusion followed hemorrhage the increase in the systolic level was correspondingly great.

To recapitulate in another way. The diastolic pressure is determined mainly by the outflow through the arteries during the diastolic pause and it is, therefore, affected mainly by the calibre of the vessels and by the rate of the heart; so that changes which affect mainly the diastolic pressure are either vasomotor or heart rate. The systolic pressure is determined mainly by the pressure with which the heart forces blood into the vessels and is, therefore, determined by the force of the cardiac contraction and by the volume of blood at the disposal of the heart; so that changes in the systolic pressure implies either variations in the force of the cardiac contractions or in the volume of the blood at the disposal of the heart.

Conclusions. The membrane manometer gives evidence of the cause of a variation of blood pressure by the form of the curve assumed. These curves are classified in the text.

Vasoconstriction is indicated by the elevation of both diastolic and systolic pressures, but the former much more than the latter, so that the amplitude of the excursion (pulse pressure) is greatly lessened.

Vasodilation is indicated by a lowering of both pressures, with the diastolic pressure lowered much more than the systolic, so that the amplitude is greatly increased. The diastolic pressure is the determining feature in both vasoconstriction and dilation.

When the heart rate is increased sufficiently to raise the blood pressure, the curve resembles vasoconstriction; while a lowered pressure from decreased rate is similar to that of vasodilation.

Following hemorrhage both systolic and diastolic pressures are lowered, but the systolic disproportionately, so that the amplitude of the excursions are lessened. The infusion of blood or saline solution increases the excursion largely by elevating the systolic level. In both hemorrhage and infusion the systolic pressure largely determines the form of the curve.

I am glad to express my indebtedness to Dr. Sollmann for many helpful suggestions, especially in the discussion of the results.

LITERATURE

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STUDIES ON THE HYDROGEN-ION CONCENTRATION IN BLOOD UNDER VARIOUS ABNORMAL CONDITIONS

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INTRODUCTION

Since the application by Höber (1) in 1900 of the Nernst hydrogen concentration chain to the measurement of the reaction of the blood, our knowledge of that subject has been greatly extended. Many modifications of the original method have been adopted by various investigators, but practically all the results published reveal the fact that the reaction of normal blood lies within comparatively narrow limits, varying according to the earlier observers between $0{,}3.10^{-7}$ and $0{,}7.10^{-7}$ (2) and according to the later and more accurate studies of Hasselbalch (3) on Mammalia at body temperature between $3{,}5.10^{-8}$ while Michaelis (4) records an average value of $2{,}75.10^{-8}$ at a temperature of from 18°C to 20°C. Both these later observers have noted that venous blood is always slightly more acid than arterial blood, due, probably, to the greater carbon dioxide tension in the former.

Even the investigations of blood in pathological conditions, where acidosis occurs, have failed to show any considerable deviation from the narrow limits already quoted, and only in diabetes, (5) (6) where deep coma has been reached, does there occur any demonstrable increase in the hydrogen-ion concentration of the blood, although oxybutyric and diacetic acids are known to be produced in not inconsiderable amounts in that disease. The only remaining conditions in which an increased acidity of the blood has been observed, where the technique is free from serious criticisms is that of narcosis, reported by Michaelis (7).

The investigations outlined in the following pages deal with changes in the hydrogen-ion concentration, noted in animals in various conditions.

METHODS

The method employed for estimating the acidity of the blood was that of measuring the hydrogen-ion concentration by means of the gas chain; and since the technique used was essentially that already published in detail by Michaelis (8) no further description need be given here. It may be mentioned however that all measurements were made on animals at laboratory temperature varying from 18°C to 24°C., and for these variations corrections were made. The quantities of blood used varying from 2.5 cc. to 3.5 cc., were diluted to 6.5 cc. with 0.85 per cent sodium chloride containing hirudin in solution. When possible the blood was allowed to drop directly into the electrode from the ear of the animal employed; in other cases a cannula was inserted in the vessel and the blood passed from this into the electrode. In every experiment duplicate readings were made.

Instead of expressing our results as the hydrogen concentration $C_H = 10^{-n}$ as has been done in the introduction, we have followed the Sörensen procedure in using throughout the hydrogen-ion exponent pH = p.

RESULTS AND DISCUSSION

In some of our earlier estimations of the hydrogen-ion concentrations of the blood it was observed that in anaesthetized animals the acidity of the blood was considerably higher than that reported for normal animals. A systematic study was then made of the blood of animals anaesthetized with chloroform, ether and nitrous oxid. For these experiments dogs and rabbits were chosen because of the comparative ease in obtaining blood from the ear blood vessels of these two animals. As previously stated the blood was bled from an incision in the blood vessel of the ear directly into the electrode; the same animal was then anaesthetized and blood again taken from the same site in a similar manner so that in every case the blood in anaesthesia could be compared with the normal blood of the same animal.

In a large number of experiments the values obtained for normal blood show a wide variation, ranging in the dog from pH = 7.64 to pH = 7.32. In the rabbit the variations are even more marked, the minimal and maximal figures obtained being pH = 7.18 and pH = 7.70, a phenomenon which will be discussed later.

Under the influence of the three above mentioned anaesthetics, when anaesthesia reaches the stage in which the reflexes are completely

abolished, the reaction of the blood measured at 20°C. may fall to pH = 7.00. Although our experimental studies of the blood reaction in anaesthesia number over twenty-five, no protocols are recorded here because the degree of acidity produced is, as far as could be ascertained, dependent solely upon the amount of the drug inspired, if corrections are made for the different weights of the various animals. Corresponding more or less closely to the degree of anaesthesia therefore, values extending from pH = 7.00 to pH = 7.60 may be obtained. The deeper the anaesthesia the more marked the acidity, and vice versa; also prolonged duration of the anaesthesia is apparently not a factor in increasing the hydrogen-ion content of the blood. Further, the increase in acidity commences as soon as the anaesthetic enters the circulation, and a diminution takes place immediately on the cessation of the administration of the drug, until 45 minutes subsequently the blood again resumes its normal hydrogen-ion concentration.

Narcosis produced by morphia, even when this drug is given in such large doses as 75 cc. of 1 per cent morphia sulphate in two hours, fails to reveal any demonstrable changes in hydrogen-ion concentration.

As it is interesting on account of the prolongation of the duration of the induced acidity, mention is made of one experiment with ethyl alcohol, where a dog whose normal blood reaction was pH = 7.47 was given by stomach tube a mixture of 60 cc. of 95 per cent alcohol in 40 cc. of water. Forty-five minutes subsequently the blood acidity was pH = 7.32 and 6 hours later, although the animal had apparently gained complete consciousness, the reaction remained at the high value of pH = 7.28.

Finally, in two cases of surgical operations in the senior author's clinic, during nitrous oxide anaesthesia, measurements showed the blood reaction to be respectively pH = 7.20 and pH = 7.22.

Not only in anaesthesia but also in other abnormal states the blood reaction was observed to become more acid. The most striking of these was fright, a phenomenon remarkably shown in the rabbit. Ordinarily if blood is taken from the ear of a rabbit, so that the animal is disturbed as little as possible, the blood shows a fairly high alkalinity. Under the influence of fright this is altered in an astonishingly short time and to a most profound extent. Another noteworthy feature is the rapidity with which the blood regains its normal reaction. The following protocols will serve to illustrate these facts:

I. Normal blood reaction of rabbit was pH = 7.67. Animal was comfortably placed in a box covered with a wire screen and frightened

for ten minutes by a barking dog. Blood immediately taken from the ear blood vessels showed an acidity of pH = 7.17. The rabbit was then allowed to rest quietly 45 minutes and again frightened, directly following which the animal was bled, and the blood reaction was found to be pH = 7.34. After being allowed to rest quietly for 30 minutes, blood examined in like manner gave a value of pH = 7.60.

II. Rabbit whose normal blood was pH = 7.60, after being frightened for 5 minutes in the manner above described, showed a hydrogen-ion concentration of pH = 6.98. Fifteen minutes subsequently the blood again gave a normal reaction. No signs of distress other than shallower and more frequent respirations seemed to accompany the increased acidity and the animal apparently suffered little inconvenience during or subsequent to the fright and 15 minutes afterward appeared to be quite normal again. It is probable however that such a process frequently repeated throughout life must leave some traces. It is indeed, most probable that the varying figures obtained for the normal blood of the rabbit noted earlier in this paper, and which have been previously reported by Hasselbalch and Lundsgaard (9) have their explanation in the above phenomenon, the repetition of which during the life of the rabbit may account for the varying pathological conditions of the blood vascular apparatus of that animal, encountered by so many investigators and which, therefore, make it such an undesirable experimental animal. Similar observations regarding such marked effects of fright have been very rarely observed by us in dogs.

Since the stimulus of fright can produce such a prompt and intense response it is to be surmised that in other emotional disturbances, similar changes in acidity would be encountered. Experiments to test this supposition were made for anger in cats, and it was found that the expression of that emotion is also accompanied by profound changes in the blood of the animal. Unfortunately the cat is not as desirable for such experiments as the rabbit. To obtain blood from the cat without general anaesthesia is much more difficult than from the rabbit, largely because in the former the blood vessels must be exposed under local anaesthesia. Further if the operation is not skillfully and quickly done so as to minimize the pain and eliminate as far as possible the excitement of the animal, the blood from the beginning assumes the high acid value indicative of emotional disturbance. The following abbreviated protocols serve to illustrate the character and results of the experiments.

I. Normal blood withdrawn by syringe from the femoral artery, exposed under cocaine, had a value of pH = 7.43. The cat was angered

by a barking dog and in blood then withdrawn from the femoral artery acidity was pH = 7.20. The animal was kept tied down and at the end of an hour the blood reaction was pH = 7.37.

II. Cat was tied down and femoral artery exposed under cocaine. Normal blood withdrawn with syringe had a hydrogen-ion concentration of pH = 7.20. Cat was then frightened for 15 minutes and blood acidity had risen to pH = 7.02. It may be noted, that in these experiments on cats the normal reaction of the blood acidity is invariably high, due, doubtless, to the effect of fright and some unavoidable distress of the animal during the experiments.

The possibility that the cause of the increased acidity in the above reported experimental data was an accelerated oxidation with the accumulation of its accompanying products in the circulation led to the estimation of the blood reaction during and immediately following insomnia, where it was thought the prolonged metabolic processes taking place might give rise to a similar condition of the blood. But tests on a series of six rabbits, housed in a warm place, abundantly supplied with food and water but kept awake for 100 consecutive hours, were quite negative on this point.

Evidence of a similar sort is also obtained by the following experiments. In pithed animals immediately following the severing of the spinal cord there is a rapid rise of hydrogen-ion concentration, due undoubtedly to the decreased lung aëration and the consequent faulty elimination of carbon dioxide, for on the establishment of artificial respiration the normal reaction of the blood is promptly restored. When in such a pithed animal where a normal hydrogen-ion concentration was being uniformly maintained by artificial respiration, violent contractions of the skeletal muscles were produced by electrical stimulation of the cut end of the cord in communication with the musculature of the thorax, abdomen and extremities, no evidence of an increased acidity of the blood was found except when the animal was dying.

One other condition in which the blood showed an increased acidity was that of shock. Only two experiments were performed but as the results in both cases were very definite, they are now outlined.

I. Blood from dog's ear had a hydrogen-ion concentration of pH = 7.61. The animal was anaesthetized with ether, the abdomen opened and intestines manipulated for $1\frac{3}{4}$ hours at the end of which time the blood pressure was exceedingly low. Thirty minutes subsequent to this manipulation and 40 minutes after the ether was discontinued, the acidity of the blood was pH = 7.11; and 60 minutes follow-

ing intestinal manipulation and 70 minutes after the cessation of the administration of the ether, the acidity had risen to pH = 6.98.

II. Reaction of dog's blood at the beginning of the experiment was pH = 7.48. Shock evidenced by a marked fall in blood pressure was produced by a manipulation of the intestines. One hour after ether was discontinued, blood from the vena cava had a value of pH = 7.08. Blood pressure at this time was so low that sufficient blood for a reading could not be withdrawn from the femoral artery with a syringe. That the increased acidity was not due to imperfect elimination of ether was indicated by the return to consciousness of both animals in from 25 to 30 minutes after the anaesthetic was discontinued.

In addition to the conditions above reported, where increased acidity of the blood obtains, is appended lastly a very interesting observation where a diminution of the hydrogen-ion concentration was noted; namely, in the blood flowing from the adrenal gland. In order to obtain blood which would contain, as far as possible, the maximal concentration of adrenal secretion, it was found necessary to clamp the adrenal vein just before it joins the vena cava, on the one side, and before it passes over the adrenal gland on the other side. The portion of the vein, between the clamps therefore, lay directly over the gland and contained only blood from that organ. When this blood was withdrawn with a syringe and measured the value of the hydrogen-ion concentration was always from pH = 0.10 to pH = 0.12 lower than the blood taken from the immediately adjoining vena cava or from that part of the adrenal vein lying distal to clamp. While this difference is not large it is a very constant feature of adrenal blood. The assumption that this increased alkalinity is due to adrenalin is supported by the fact that the addition of adrenalin to blood serum, whose hydrogen-ion concentration is known, lowers its acidity and the diminution corresponds to the weight of adrenalin added when this is below the amount necessary for saturation. Thus if adding a definite amount of this substance, which as has been shown by Aldrich (10) and others to be a very strong base, causes a certain increase in the hydroxyl ion concentration, then when twice or four times that amount is added the increase in alkalinity is multiplied by two and four respectively. Since at no time was it possible, with the apparatus used, to obtain blood from the vena cava, in the immediate vicinity of the opening of the adrenal vein which was appreciably more alkaline than blood from any other part of the vena cava, the adrenalin apparently causes a measurable modification of the blood in a very limited area. Moreover, that the influence of the

adrenalin on the circulatory fluids is local in character is further evidenced by the fact that the removal of the gland caused no change whatever in the reaction of the blood until the animal was moribund. Although blood from the pancreas, liver and thyroid, as well as from the internal and external jugular veins was compared with blood from the vena cava of the same animal, no evidence of any variation in the hydrogen-ion concentration could be obtained. Further the removal of these organs singly or in combination caused no change in the reaction of the blood.

The data presented in this paper proves conclusively that a very marked increase in the hydrogen-ion concentration may occur under certain abnormal conditions, and that the existence of this high acidity of the blood is not incompatible with life. As to the cause of the phenomenon and regarding its significance, the authors feel the present data to be too limited to warrant much speculation. Concerning the first point, however, one or two suggestions may not be amiss. The immediate and intense response of the blood to emotional stimuli by a marked rise in the hydrogen-ion concentration with the corresponding alteration of the character of respiration, and the rapid disappearance of these on the removal of the exciting cause, indicate that under these circumstances the carbon dioxide is a major factor; it is obvious however that all increased acidity of the blood cannot be ascribed to this source. This explanation does not suffice for shock and anaesthesia, since in the former the carbon dioxide in the blood is markedly diminished as has been shown by Henderson (11), and in the latter the carbon dioxide in the blood is increased according to the researches of Buckmaster and Gardner (12). Indeed in such a composite fluid as blood, containing so many complex chemical compounds, it is quite conceivable as has been suggested by Robertson (13) that the amphoteric character of certain proteins must be of extreme importance in regulating the acidity of the blood.

SUMMARY

1. The hydrogen-ion concentration of the blood during certain emotional disturbances, such as fright in rabbits and dogs, and anger in cats, is markedly increased and at a temperature of 20°C., frequently reaches an acidity corresponding to pH = 7.00. This is probably due to increased carbon dioxide tension.

2. In anaesthesia caused by ether, chloroform and nitrous oxid the hydrogen-ion concentration may be increased to the same ex-

tent. The change in acidity begins when the inspired anaesthetic commences to react with the blood, and depends, approximately, on the degree of the anaesthesia. The restoration of the normal reaction of the blood is completed in 45 minutes after the administration of the drug is discontinued.

3. In two cases of shock the acidity of the blood was very much increased.

4. The blood flowing from the adrenal gland is always more alkaline than venous blood elsewhere in the body. This increased alkalinity is local, not extending to any appreciable extent beyond the immediate vicinity of the adrenal vein, and is due to the dissolved adrenalin which it contains.

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THE ORIGIN OF ANTITHROMBIN

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INTRODUCTION

It is a well-known fact that Witte's peptone when injected intravenously into dogs produces in their blood an anti-coagulating substance which causes blood withdrawn from such animals to remain fluid for long periods. The plasma from peptonized animals has the power to delay or inhibit the coagulation of normal blood in vitro.

Many workers have studied peptonized animals with a view of finding the origin of the anti-coagulating substance, among them being Delezenne (1), Nolf (2), Doyon (3), and Popielski (4). All agree that the liver is the most important site; in fact, with the exception of Popielski they feel it to be the only site. The last named author thinks the intestine and extremities play a large part.

Almost all experiments on the origin of antithrombin have been carried out by means of peptone injections and it has been found that in animals with the liver cut out of the circulation peptone is without effect on coagulation. The liver then seems essential to the production of the anti-coagulating substance which we shall call antithrombin according to Howell's theory (5).

Just in what way peptone acts on the liver is a disputed point but all workers agree that Witte's peptone itself has no action. It is some substance formed in the blood which stimulates the liver to produce antithrombin. Delezenne believed the destruction of leucocytes to play the whole part. Nolf's work does not substantiate this. Both Nolf and Popielski believe the liver endothelium produces antithrombin but this point is not as yet adequately proven. No attempt will be made in this paper to discuss the various theories of the method of peptone action but the above brief summary serves to indicate that the liver is the organ most concerned.

Up to the present time almost all determinations of antithrombin have been made by determining the effect of the peptone plasma on whole blood. This of course is a very rough method and serves only to show a great increase in antithrombin.

According to Howell's theory of coagulation antithrombin is a normal constituent of the blood. By its antagonizing action to prothrombin it prevents the latter from being converted to thrombin and tends to neutralize the action of any thrombin that may be formed. In other words it is the normal excess of antithrombin which prevents intravascular clotting. If antithrombin is normally present in the blood, and if, as previous workers have shown, it is formed in the liver, it would seem likely that in a normal animal blood taken from the hepatic vein would show a greater amount of antithrombin than blood from other parts of the body.

Doyon (6) has reviewed the numerous articles on the coagulation time and fibrinogen content of blood so taken and finds the results so greatly at variance that no conclusions are possible. He concluded from work of his own that such blood may coagulate more quickly than carotid blood. Nolf found that whole blood introduced into a living washed liver clotted normally.

We have taken up the study of blood after its normal passage through various organs and after stasis in the same organs.

TAPPING OFF OF ORGANS

In order to determine how the antithrombin content of the blood may vary in different parts of the body a series of six experiments were done, the results of which are tabulated below.

Method. A dog was etherized, after previous administration of morphia, and a tracheal cannula inserted for continued etherization and artificial respiration. Dissecting down on the jugular vein a specimen of blood was drawn from it as a control, ten to twenty minutes before a head thorax circulation was established. In all but Experiment II ligatures were then placed in the following order: around the inferior vena cava in the thorax close to the diaphragm, around the inferior cava directly below the liver, and around the aorta directly below the diaphragm. In Experiment II the order of the ligatures at the liver was reversed, first below and second above.

It took about ten minutes to place these ligatures after the vessels were exposed. The ligatures were allowed to remain until after the experiment was completed.

Specimens of blood were drawn as rapidly as possible after the stasis was produced, usually in the following order: first, from the portal vein, second, from the inferior vena cava below the lowest ligature, and third, from the hepatic vein. In Experiments III and V the order was, the inferior cava, the hepatic vein and the portal vein. It was about *ten* minutes from the time the last ligature was tied until the last of the above samples of blood was obtained. The antithrombin determinations of these specimens are recorded in the table as "before stasis." To be sure, a few minutes of stasis of the blood had occurred before these samples were obtained, but they represent blood before prolonged stasis had taken place. After the blood below the diaphragm had remained stagnant and a head thorax circulation had existed for varying lengths of time, 30–70 minutes, as recorded in each instance in the table, other specimens of blood were drawn from the same regions for comparison.

Samples of blood were drawn from the splenic and renal veins in three experiments before the ligatures creating a head thorax circulation were tied; then the arteries and veins of these organs were clamped and after an interval of stasis blood was again drawn to be compared with other specimens.

The specimens of blood to be examined were drawn with a Luer syringe previously rinsed with salt solution. A definite volume of this blood was put into a tube with a constant amount of 1 per cent sodium oxalate in 0.9 per cent sodium chloride solution and well mixed, centrifuged for 20 minutes, and the plasma pipetted off, heated to 60°C. to precipitate the fibrinogen and destroy prothrombin, filtered and examined for antithrombin by the method described by Howell (7) and discussed by Minot and Denny (8).

In brief the test used was as follows: 1 drop of the antithrombin solution (after heating to 60°C. and filtering) from each specimen was mixed in a tube with the varying amounts of a solution of thrombin. After these two substances had been in contact for a given interval, a constant amount of a fibrinogen solution was added to each tube so that the clots formed in a period of time suitable to distinguish differences. The time taken for clotting was upon the first appearance of a clot, that which clotted first having the least antithrombin and that last the most. At least four suitable series of determinations on the different specimens were made. To compare the specimens of any single experiment, the time that their antithrombin allowed thrombin and fibrinogen to clot was sufficient.

But in order to compare those of different experiments time could not be satisfactorily used, owing to differences in the reagents, so that antithrombin is expressed as the antithrombin factor (8). The average time of clotting of a series of suitable determinations of a specimen was divided by a similar figure obtained for a control. The control in these experiments was the specimen from the jugular vein before any stasis was produced and its factor was taken as unity.

By referring to the table it will be noticed that in the six different experiments the antithrombin in the venous blood of the jugular or superior vena cava before and after a head thorax circulation remained about the same.

The two observations of splenic blood before stasis showed less antithrombin than the jugular blood. The three specimens of splenic vein blood after stasis all gave a plasma in which some definite hemolysis had taken place. It has been shown that large amounts of fatty acids occur in the spleen and this may be the reason that the blood standing in the spleen is hemolyzed. Except for one specimen of renal plasma, none of the other specimens showed any hemolysis. It was felt that the hemolysis in this renal specimen might have been due to the fact that the syringe contained water and was not washed out with salt solution before the blood was drawn.

Antithrombin, being "neutralized" by thromboplastin, cannot be determined satisfactorily in a plasma in which hemolysis is apparent, so that the figues for antithrombin after stasis of blood in the spleen are probably lower than they should have been owing to the presence of thromboplastin liberated from the hemolyzed corpuscles. The two observations of antithrombin in renal vein blood before stasis were similar to the control with a slight tendency to rise after stasis.

Portal blood before prolonged stasis was studied in five experiments; in four it contained less antithrombin than the control, in one a greater amount. In the three observations after an interval of stasis from 32–60 minutes there was a slight rise.

Inferior vena cava blood (taken from below the lowest ligature) in the four experiments in which it was studied showed less antithrombin than the control, with a definite rise after an interval of 32 to 48 minutes of stasis in three instances, and essentially no change in the fourth.

Hepatic vein blood before prolonged stasis showed an antithrombin content essentially like the control blood. Just before the specimen to be tested after stasis of 30 to 70 minutes was obtained, varying amounts of blood were drawn off from the vein and discarded so as to

obtain blood that had been in actual contact with the liver cells. Owing to the short length of the hepatic vein it was easy to draw blood from the cava that had stood between the clamps above and below the liver instead of obtaining hepatic vein blood. To obviate this difficulty in the last three experiments just before the hepatic blood after prolonged stasis was drawn, a clamp was placed across the cava above the liver, just below the point where the hepatic vein enters it.

After prolonged stasis of blood in the liver there was in the last four experiments a very marked rise in the antithrombin, a much greater amount than in blood from other sources. In the first experiment there was a slight rise and in the second a fall. Why there was not a greater rise in Experiment 1 and a fall in Experiment 2 is not clear, but it is possible that the blood obtained had not been stagnating deep in the liver tissue.

Not only does the antithrombin show a distinct increase after stasis of blood in the liver, but the clot formed by the thrombin and fibrinogen in the presence of the antithrombin from this source was of a weak character such as occurs when one has increased antithrombin.

The portal blood and the blood from the inferior cava and spleen, obtained before stasis, had less antithrombin in most instances than that from the jugular. The renal blood on the contrary had about an equal amount, as did the hepatic. Blood taken after stasis in any organ showed in most instances a slight rise in antithrombin. This was particularly evident in the inferior cava below the lowest ligature, but in no case was found the great rise observed after stasis in the liver.

In Experiment VI the time of clotting of the recalcified oxalated plasma was determined. Precautions were taken to find the optimum amount of calcium for this clotting and the time obtained designated as the prothrombin time. It constitutes a test of the relative efficacy of the prothrombin present. To a series of tubes each with 5 drops of plasma from each specimen 2, 3, 4 and 5 drops of 0.5 per cent $CaCl_2$ solution were added and the time of clotting noted. The tubes with 3 drops of calcium were the first to clot in all instances, i.e., had the optimum amount of calcium. The shortened time occurring with plasma from the splenic blood after stasis in this organ, is undoubtedly due to the hemolysis and the consequent liberation of thromboplastin as we have frequently noticed on other occasions.

Prothrombin times are as follows:

Jugular vein before stasis 5 minutes.

Jugular vein after stasis 5 minutes.

Splenic vein after stasis 3 minutes.
Renal vein before stasis 5 minutes.
Renal vein after stasis 5 minutes.
Portal vein before stasis 5 minutes.
Portal vein after stasis 5 minutes.
Hepatic vein before stasis 5 minutes.
Hepatic vein after stasis 35 minutes.

A striking delay in the prothrombin time occurred with the hepatic blood after stasis. This delay is probably wholly due to the increased antithrombin content, although possibly there was also a decreased amount of prothrombin.

Examination of arterial and venous blood taken from the heart has not shown any difference in either antithrombin or prothrombin content.

PERFUSION OF LIVER

Perfusion of the liver with mixtures of blood and peptone, plasma and peptone, etc., has been shown by Delezenne (1), Nolf (2) and others to produce antithrombin. Both of these authors have perfused the liver with defibrinated blood alone and Delezenne came to the conclusion that such blood acquired a very slight anticoagulating power. Nolf also obtained negative or doubtful results.

Doyon (9), by attaching the carotid artery of one dog to the portal vein of another which had been bled to death found that on emergence from the inferior cava the blood was slow to coagulate and retarded the clotting of normal blood. Positive results were obtained only with young, fasting dogs. With the exception of the above work we have been unable to find in the literature any successful demonstration of a formation of antithrombin by perfusion of the liver with a normal circulating medium without drugs added to the perfusing material.

We have perfused the liver with defibrinated blood in the following manner:

Method. A large dog was bled to death while under ether and the blood defibrinated. Part of this was diluted 1 to 2 with normal salt solution and used for irrigation and the rest was used to perfuse the liver of a second dog.

Dog II was given morphia and ether, the abdomen opened and the portal vein exposed. A large cannula was placed in the portal vein and the inferior cava quickly clamped above its tributaries, close to the liver. The hepatic artery was also clamped at this time.

	EXTERNAL JUGULAR OR SUPERIOR VENA CAVA			SPLENIC VEIN			RENAL VEIN			PORTAL VEIN			INFERIOR VENA CAVA BELOW LOWEST LIGATURE			HEPATIC VEIN			
	Antithrombin before starting experiment	Antithrombin at end of experiment	Duration of the head thorax circulation	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Amount of blood withdrawn before specimen was taken
																			cc.
Experiment I	1.0	0.93 0.85	8′ 40′		0.64	5′		1.07	3′	1.3						1.07	1.22	30′	20
Experiment II	1.0	1.16	60′							0.66	0.77	32′	0.73	0.71	40′	1.00	0.83	50′	15
Experiment III	1.0	1.0	30′							0.92			0.77	1.23	32′	1.07	1.80 1.76	35′ 37′	18 25
Experiment IV	1.0	1.0	35′	0.85	0.80	25′	1.0	1.2	30′				0.78	1.30	40′	1.07	1.85 2.02	43′ 45′	20 30
Experiment V	1.0	1.0	50′							0.68	1.00	35′	0.68	1.06	38′		2.18 1.70 2.22	40′ 42′ 44′	12 20 30
Experiment VI	1.0	1.2	70′	0.80	0.80	30′	1.2	1.3	40′	0.9	1.20	60′				1.00	3.00 3.80	65′ 70′	24 30

While one operator was doing this the other had opened the chest and introduced a cannula into the inferior cava just above the liver and washing with diluted blood was immediately started through the portal vein. As soon as the washing fluid had been run through perfusion was started with defibrinated blood. The time during which the liver had no circulation varied from 4 to 7 minutes.

The apparatus used for perfusing was in most cases very simple. It consisted of a large tank filled with water. In this was placed the reservoir for the perfusing and washing fluids from which tubes lead to the inflow cannula in the portal vein. The flow was by gravity. A thermometer was introduced at a point just before the entrance into the liver and the perfusing fluid was kept at about 38°C. by regulation of the temperature in the large water bath.

The outflow was caught in a container kept at about 38° by means of a water bath. The outflow was whipped, when defibrinated blood was used as a perfusate, since it was found that the first specimens coming through after washing showed some tendency to clot. Defibrination was continued only during the first part of the experiment and was necessary probably because the liver had not been washed entirely free from blood. In some experiments oxygen was bubbled into the outflow container and in one an artificial lung devised by Dr. Hooker was used. Oxygenation seemed to make little or no difference since those experiments in which no attempt was made to oxygenate gave similar results. The outflow was poured back by hand into the reservoir above and thus allowed to circulate many times.

Perfusion was carried on under a pressure of 90 to 120 mm. of mercury. Although there was a certain amount of loss in the amount of the perfusing liquid the latter was not concentrated enough to account for any significant increase in antithrombin content. The method, however, has the objection that the system was not closed.

Specimens were taken of the perfusate before perfusion was started and at intervals during perfusion. These were taken in 5 cc. lots and mixed with 0.7 cc. of 1 per cent oxalate in normal saline. After centrifuging, antithrombin tests were done on the serum by the method of Howell previously described.

Four experiments were done.

Experiment I. Perfusion with Ringer's solution.

Dog—Morphia and ether. Cannulas placed as described. Liver washed with 3 liters of Ringer's solution until outflow was clear.

Perfusion then started with about 200 cc. of Ringer's solution. This was clear at the start but quickly became bloody. Whole system cleaned out and started again with fresh Ringer's. Perfused in first case for about one hour and in the second for one and a half hours. The latter became bloody and a third perfusion was done with fresh Ringer's after washing. After one hour and forty-five minutes perfusion was again bloody. The specimens taken during the last perfusion showed a heavy precipitate on heating to 60° which was not fibrinogen and was evidently protein from the dying liver. None of the seven specimens taken during the perfusion showed any more antithrombin than might be explained by the blood which had been picked up during the repeated passage through the liver.

Experiment II. Perfusion with defibrinated blood.

Preparation as described.

Liver washed out with 100 cc. defibrinated blood plus 200 cc. normal saline. Perfusion started with 570 cc. defibrinated blood after a control specimen had been taken. This is labeled I in the table. Specimens taken 30, 40, 50, 58 and 64 minutes after the start of perfusion are labeled II, III, IV, V, and VI.

All specimens centrifuged at high speed for 20 minutes, serum pipetted off, heated to 60° and filtered. On heating none showed any precipitate.

One drop of the heated and filtered serum (antithrombin) was added to the thrombin in varying amounts and after an interval of 15 minutes 10 drops of fibrinogen were added.

Antithrombin determination

THROMBIN	I	II	III	IV	V	VI	SPECIMEN NUMBER
4 gtt.	18′	30′	37′	41′	46′	48′	Figures represent time in minutes which it took for clot to form after addition of fibrinogen.
5 gtt.	12′	22′	22′	27′	38′	40′	

The two series correspond well and show a very marked gradual increase in antithrombic power.

Experiment III. Perfusion with defibrinated blood.

Large dog bled to death and cannulas inserted into the portal vein and inferior cava of another dog in the usual way. Liver washed with 100 cc. defibrinated blood plus 200 cc. normal salt solution.

Perfusion started after control specimen had been taken and 20 minutes later Specimen II was obtained.

Just after this was taken a rather large amount of water was spilled accidentally into the outflow jar. This of course produced great hemolysis as well as dilution in the circulating blood and the effect in lowering antithrombin will be seen in the table.

Specimens taken 65, 95 and 108 minutes after start of perfusion are labeled III, IV and V in the table.

On centrifuging, those specimens taken after the accident were much more hemolyzed than those before it. Antithrombin tests were run in the usual way.

Antithrombin determination

THROMBIN	I	II	III	IV	V	SPECIMEN NUMBER
5 gtt.	21′	21′	7′	14′	16′	Figures represent time in minutes which it took a clot to form after addition of fibrinogen.
6 gtt.	10′	11′	7′	10′	12′	

No. III represents the first specimen taken after spilling water into the blood. Nos. IV and V show a gradual increase in antithrombin content although the antithrombin never reached the concentration that it had at the beginning.

Experiment IV. Perfusion with defibrinated blood.

This experiment was carried on in the usual way except that the outflow from the liver was connected with an artificial lung devised by Dr. Hooker. This ensured thorough oxygenation of the blood. The liver was washed with mixture of 300 cc. normal salt solution and 150 cc. defibrinated blood. Perfusion started after taking control, Specimen I. Specimens II, III, IV and V taken 30, 59, 70 and 83 minutes respectively after the start of perfusion. After Specimen V was taken 1.5 gm. peptone in solution was added to the perfusing material (about 400 cc.). Specimens taken 15, 24 and 34 minutes after addition of peptone are labeled VI, VII, and VIII in the table.

On centrifuging, all specimens showed hemolysis as usual, the degree increasing somewhat as the experiment went on.

Antithrombin determination

THROMBIN	I	II	III	IV	V	PEPTONE ADDED	VI	VII	VIII	SPECIMEN NUMBER
5 gtt.	10′	13′	13′	19′	No clot in 30′		No clot in 30′			
6 gtt.	8′	8′	8′	9′	Very poor clot 15′		Poor clot in 21′	26′	40′	

From this table it will be seen that antithrombin was not acquired by the perfusing fluid for about 1 hour, Specimen IV being the first to show any change. Specimen V shows a well marked increase. The specimens taken after addition of peptone show a still further increase in antithrombin content but not to the extent which one would expect

in a fresh liver perfused with a blood and peptone mixture. Probably much of the antithrombin had been taken up from the liver in this case before the peptone was added to the perfusate.

These experiments show that the frequent passage of defibrinated blood through the liver results in a gradual increase is the antithrombin content of the perfusate.

Specimens of defibrinated blood were obtained from perfusions of the spleen, head and hind leg done by other workers in this laboratory, and although in some of these cases the perfusions were carried on much longer than in our experiments with the liver, we were unable to demonstrate any marked increase in antithrombin; in fact, usually the amount was decreased. Three perfusions of the head showed in each instance a very slight increase of antithrombin.

The decrease in antithrombin was probably due to hemolysis and deserves a word in connection with our experiments.

The centrifuged serum from defibrinated blood always shows a certain amount of hemolysis. In perfusing for any length of time the amount of this hemolysis increases distinctly up to a certain point. Since hemolysis liberates an active thromboplastic substance some of the antithrombin in the perfused blood must have been neutralized and therefore the increase in antithrombin which we actually found after perfusion of the liver probably did not represent the total increase obtained. If we could have done away with this factor of hemolysis our results would probably have been more striking.

ATTEMPTS AT STIMULATION OF LIVER

Having shown that stasis in the liver causes an increase in antithrombin and that perfusion with defibrinated blood likewise causes an increase we next attempted to stimulate the liver in various ways.

Doyon (10) has attempted this also and claims success as a result of intravenous injection of bile and bile salts. He found that bile salts injected intravenously could cause an incoagulable blood, and that doses which were ineffective in the general circulation were capable of delaying coagulation when injected into the mesenteric veins. He did not show that the long coagulation time was due to an increased antithrombin.

The substances which we have used were all injected into the mesenteric veins, and all the dogs used were starved 24 hours or longer. These experiments will not be given in detail because they were entirely negative.

Experiment 1. Effect of bile salt. Dog, weight 7 kg.

9.5 cc. of 10 per cent solution of sodium glycocholate was injected into the mesenteric vein. In 10 minutes the coagulation time had gone from 40 minutes (normal) to one hour and twenty minutes and remained in this vicinity for three hours.

All specimens taken after injection showed a very marked hemolysis and the antithrombin was uniformly reduced as compared with the control specimen. Prothrombin not changed to any marked degree. We felt that larger doses might cause greater delay in coagulation time but that the increasing hemolysis would make it impossible to say if it were due to an increased antithrombin.

Experiment 2. Effect of bile by mouth.

A dog, weight 8 kg., having had control specimen of blood taken, was given 16 gm. of dried ox bile in 200 cc. water by stomach tube. Allowed to come out of ether. Vomited a little (40–50 cc.).

Two hours and fifteen minutes after giving bile another specimen of blood was taken. 17 gm. of ox bile in 150 cc. water then given by stomach tube. Specimens taken one and two hours later showed no hemolysis, no change in coagulation time and no change in prothrombin and antithrombin content.

Experiment 3. Effect of secretin.

Dog, weight 7 kg. Morphia and ether.

10 cc. of a secretin solution prepared according to Bayliss and Starling (11) injected into the mesenteric vein. Specimens taken 10 minutes after, showed coagulation time of 56 minutes against the control time of 28 minutes—20 minutes after injection coagulation time was 29 minutes. 9 cc. more injected and specimens taken 16 and 24 minutes later, showed a coagulation time of 41 and 55 minutes respectively.

Large amounts of secretin introduced into the mesenteric veins with a burette failed to further lengthen the coagulation time.

No pancreatic fistula having been made, it is not certain that the secretin used was active. There was a slight lengthening of the coagulation time after the first two injections but on the whole the experiment must be considered negative. There was no change in the antithrombin content of the numerous specimens taken after the various injections.

Experiment 4. Effect of nerve stimulation.

Dog, weight 8 kg. Morphia and ether. Control specimens taken for coagulation time and antithrombin. Abdomen opened and coeliac plexus exposed. Electrode passed under the ganglion and stimulation applied with a rather strong current from an induction coil. Duration of stimulation 20 seconds with an interval between of 40 seconds. The procedure was continued for one hour, specimens being taken at 15, 35 and 50 minutes from the start of stimulation.

These specimens showed no change in coagulation time or antithrombin content. The hepatic branches of the plexus were next isolated and stimulated with the result that no change was obtained either in coagulation time or antithrombin.

Experiment 5. Effect of injection of thrombin.

Dog, weight 8 kg. Morphia and ether.

Injection of 0.4 gm. of pure thrombin in which there was some salt (this solution was very active in clotting fibrinogen) into the mesenteric vein. In a specimen taken 12 minutes after injection there was lengthening of coagulation time

from 30 minutes (normal) to 43 minutes and an increase in antithrombin from 10 minutes to 17 minutes.

Davis (12) found an increase in antithrombin by injecting thrombin in very large amounts into the general circulation. Here we see a small amount into the mesenteric vein giving a slight but definite effect.

PHOSPHORUS POISONING

Antithrombin as Influenced by Phosphorus Poisoning in Dogs

If the liver is the site of antithrombin formation it would seem probable that a liver destroying substance such as phosphorus would lower the antithrombin content of the blood. It has been shown by many Jacoby (1900); Loeb (1903); Morawitz (1905); Doyon (1906); Nolf (1908); and Whipple and Hurwitz (13) that the fibrinogen of the blood in phosphorus poisoning is greatly diminished or almost absent.

We have studied the blood of five dogs with varying degrees of phosphorus poisoning, all of which showed extensive liver destruction at autopsy. The specimens of blood were kindly given us by Drs. Goodpasture and Marshall, to whom we wish to express our thanks.

Antithrombin determination

	PHOSPHORUS DOG	NORMAL DOG	ANTITHROMBIN FACTORS
Dog I	9′	14′	0.64
" II	8′	15′	0.53
" III	11′	25′	0.44
" IV	2′	16′	0.12
" V	2′	13′	0.15

Figures represent time of formation of a clot in the usual antithrombin test. It will be remembered that the normal antithrombin factor is approximately 1.

Dog IV was the most severely poisoned and shows the greatest diminution in antithrombin; in fact, almost a complete absence of any anticoagulating power. All specimens showed by rough tests a diminution in fibrinogen.

These results tempt speculation as to what part of the liver is concerned in the formation of antithrombin. It will be remembered that Nolf and Popielski are inclined to think that the endothelium in the liver is responsible.

While it cannot be stated that phosphorus does not injure the endothelium of the liver there can be no doubt of its destructive action upon the liver cells and we suggest from our results that antithrombin may very likely be a true liver cell product. However, further work on this subject, with careful histological study, must be done in order definitely to demonstrate this point.

Numerous clinical cases of liver disease which we have studied have shown no constant variation in antithrombin content of the blood. A few with low fibrinogen have shown a low antithrombin. Some cases having a normal antithrombin showed very extensive destruction of liver substance at autopsy but it is a fairly well recognized fact that only small amounts of active liver tissue are necessary to carry on the functions of the liver.

CONCLUSIONS

1. Antithrombin is formed in the liver.
2. Venous blood taken from the liver, spleen, kidneys and intestines shows no appreciable difference from jugular vein blood in antithrombin content.
3. When stasis is produced in these organs the liver blood alone shows a definite increase in antithrombin.
4. Perfusion of the liver with defibrinated blood causes an increase of antithrombin in the perfusate.
5. Perfusion of the head, spleen and hind leg shows no marked increase and generally a decrease in antithrombin content.
6. Attempts to stimulate the liver to production of antithrombin by means of bile, bile salts, secretin and electrical stimulation were negative. Injection of small amounts of thrombin into the portal circulation caused a slight rise in antithrombin.
7. Dogs with phosphorus poisoning and liver destruction show a very marked decrease in antithrombin content of the blood as well as diminished fibrinogen.

We wish to express our gratitude to Professor Howell for invaluable advice and suggestions and to thank Dr. D. R. Hooker for material from his experiments and for help with our perfusion work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXV. A Note on the Chemistry of Normal Human Gastric Juice

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The gastric juice employed in this work was obtained from Mr. V., our gastric fistula case with complete cicatricial stenosis of the oesophagus of 20 years' standing. The gastric juice is therefore at no time mixed with saliva. The appetite juice is thus secreted during the mastication of the food and some data relative to the secretion of appetite and hunger gastric juice in Mr. V., as a type of normal adults, have already been reported (1). A few samples of appetite gastric juice was secured from a second gastric fistula case with partial cicatricial stenosis of the oesophagus. Samples of appetite and of hunger gastric juice were also secured from one of the authors (A. J. C.) by a method that will be referred to later. A few controls are also noted on dog's gastric juice, that is, the secretion from the accessory stomach pouch prepared according to Pawlow. Most of the present work refers to appetite gastric juice. A few determinations were made on samples of the hunger gastric juice. The reader is referred to our previous report for an account of the continuous or hunger gastric secretion.

I. *The solids of the gastric juice.* 100 cc. of six different lots of appetite gastric juice of Mr. V. were evaporated on water bath and then dried to constant weight in an oven at 112° C. The incineration was made at dull red heat so as not to drive off the potassium chloride, which according to Rosemann is more abundant in gastric juice than the sodium chloride (2). The results are given in Table I. On three of these lots of appetite juice the specific gravity, the freezing point, and the total acidity (phenolthalein) was also determined. The one determination on hunger gastric juice represented many separate collections of juice, as no juice was included in this lot if it was secreted by

the empty stomach at a greater rate than 3–4 cc. per hour. For the purposes of comparison the analyses of two lots of the juice or fluid found in the stomach free from food one hour after putting 200 cc. of water into the stomach are also given in Table I. It will be seen from Table I that the total solids of the appetite juice vary from 0.48 gr. to 0. 58 gr. per 100 cc., of which 0.34 gr. to 0.47 gr. is organic, and 0.11 gr. to 0.14 gr. inorganic material. The hydrochloric acid is, of course, expelled in the process of evaporation and drying of the gastric juice residue.

The hunger gastric juice of Mr. V. is distinctly higher than the appetite juice in total, and in organic solids. It appears also to be higher than the average appetite juice in inorganic solids.

The gastric juice or fluid in the stomach free from food one hour after ingestion of 200 cc. of water is distinctly more dilute than the

TABLE I

The composition of pure human gastric juice. The solids of appetite gastric juice, hunger juice, and content of "empty" stomach per 100 cc. of juice.

	APPETITE GASTRIC JUICE						HUNGER GASTRIC JUICE	CONTENTS OF THE "EMPTY" STOMACH	
	I	II	III	IV	V	VI		I	II
ecific gravity..	1007	1009	1006		1008			1004	1006
Δ	−0.550	−0.580	−0.530					−0.31	−0.45
idity (total)...	0.45	0.48	0.42				0.34	0.22	0.33
tal solids.....	0.557 gr.	0.606 gr.	0.480 gr.	0.571 gr.	0.580	0.544	0.667 gr.	0.360 gr.	0.450 gr.
ganic.....	0.420 gr.	0.466 gr.	0.341	0.452 gr.	0.470	0.430	0.521 gr.	0.260 gr.	0.350 gr.
organic.....	0.137 gr.	0.140 gr.	0.139	0.119	0.110 gr.	0.114 gr.	0.146 gr.	0.100 gr.	0.100 gr.

appetite juice, although it may approach the concentration of the latter in cases where the rate of the continuous secretion is considerable (Lot II).

The above figures on Mr. V.'s appetite gastric juice are slightly higher than those given by Sommerfeld (3) for the gastric juice from a ten-year-old girl, namely, 0.40 gr. to 0.47 gr. Sixty years ago Schmidt (4) reported on the gastric juice of a human gastric fistula case, the oesophagus being partly patent. He found total solids 0.58 per cent, of which 0.32 per cent was organic, and 0.26 per cent inorganic. But Schmidt did not work with pure gastric juice This is evident from his method of obtaining the juice, as well as from the fact that the acidity of the juice was only 0.20 per cent, which is less half that of normal human gastric juice. The figure of 0.26 per cent for inorganic solids is probably also too high Albu (5) reports one experiment on

a patient with hypersecretion finding the percentage of solids only 0.24 gr. practically all of which (0.23 gr.) was inorganic salts. Albu also reports one determination on normal human gastric juice (pure appetite juice) in which the inorganic solids were 0.18 per cent; the organic solids are not given.

Our results on Mr. V. agree closely with most of those reported for the gastric juice of dogs, as the following figures will show:

Observer	*Total Solids per cent*	*Organic per cent*	*Inorganic per cent*
Pawlow and Schoumow-Simanowksi (6)	0.47		
Konovaloff (7)	0.48		
Schoumow-Simanowksi (8)	0.528	0.393	0.135
Rosemann (2)	0.427	0.294	0.132
Nencki and Sieber (9)	0.306		

The total concentration of organic and inorganic substances is therefore about the same in the normal gastric juice (appetite juice) of man and dog. The very low solids (in one case only 0.16 per cent) reported by Nencki and Sieber are clearly exceptional and must be due to some unusual condition of their animal.

II. *The specific gravity of the gastric juice.* The specific gravity of Mr. V.'s appetite gastric juice varies between 1006 and 1009 with an average of 1007. This is the average of twenty tests on an equal number of gastric juice samples. It will thus be seen that 1009 is an exceptional concentration.

The specific gravity of the hunger juice is certainly higher than that of the appetite juice, since the total solids are higher, but no actual measurements were made on the hunger juice. The specific gravity of the fluid or juice found in the empty stomach thirty to sixty minutes after ingestion of water varies from 1003 to 1006, with an average of 1004.

The specific gravity of the appetite gastric juice of the dog, as reported by Schoumow-Simanowksi, Konowaloff, Friedenthal (10), and Rosemann varies from 1002 to 1007, with an average of 1004. This low average figure is probably due to the fact that in most of the experiments the gastric juice was collected for several hours after only a few minutes sham feeding. There is evidence that the percentage of solids in the gastric juice is greatest during the first hours of appetite or digestion secretion. The concentration of the dog's appetite gastric juice during the first twenty minutes of secretion will in all probability be found identical with that of man for the same period.

III. *The osmotic concentration of the gastric juice.* Our data on the osmotic concentration of the gastric juice are brought together in Table II. The appetite juice lowers the freezing point, −0.55° C. to −0.62° C.; the hunger juice from −0.47° C. to −0.52° C.; the fluid of the empty stomach −0.21° C. to −0.41° C. The juice found in the empty stomach an hour after ingestion of water exhibits the

TABLE II

The osmic concentration of normal human gastric juice (Mr. V.) The total acidity of the samples of gastric juice were determined with phenothalein as the indicator

FLUID IN "EMPTY" STOMACH		HUNGER JUICE		APPETITE JUICE	
Total acidity	Δ	Total acidity	Δ	Total acidity	Δ
per cent		*per cent*		*per cent*	
0.33	−0.38	0.40	−0.51	0.53	−0.58
0.26	−0.33	0.39	−0.52	0.52	−0.61
0.35	−0.42	0.36		0.45	−0.57
0.26	−0.30	0.35	−0.51	0.53	−0.60
0.30	−0.39	0.32	−0.50	0.52	−0.61
0.27	−0.38	0.36	−0.53	0.47	−0.59
0.18	−0.25	0.29	−0.49	0.42	−0.55
0.33	−0.38	0.28	−0.45	0.47	−0.60
0.17	−0.21	0.30	−0.47	0.49	−0.57
0.21	−0.27	0.41	−0.52	0.53	−0.60
0.17	−0.25	0.36	−0.49	0.45	−0.59
0.25	−0.32	0.41	−0.53	0.50	−0.61
0.26	−0.33	0.40	−0.52	0.49	−0.60
0.27	−0.37	0.32	−0.50	0.51	−0.57
0.18	−0.28	0.25	−0.47	0.46	−0.62
	−0.33			0.51	−0.60
	−0.37			0.51	−0.60
				0.47	−0.57
				0.48	−0.55
				0.49	−0.59
					−0.62
					−0.62

greatest fluctuations in osmotic pressure, the appetite and the hunger juice being very constant. The hunger juice has, on the whole, a lower osmotic concentration than the appetite juice. The total acidity of the hunger juice is also uniformly lower.

The above figures for the appetite gastric juice of Mr. V. are practically identical with those reported on the pure gastric juice of other

human fistula cases. Sommerfeld (in a ten-year-old girl) found the freezing point to vary from −0.47° to −0.65° C.; Kaznelson (11) (25-year-old girl) reports a variation from −0.46° to −0.54°. Umber (12) reports two tests on the gastric juice (pure) of a 59-year-old man with cancer, finding a variation of −0.15° C. to −0.82° C. Assuming that Umber's determinations are correct, the gastric juice of this cancer patient was clearly not normal. We question whether the normal stomach can secrete a juice with an osmotic concentration so much greater than the blood as the figure −0.82° C. demands. The reader will note that the figures of Sommerfeld and Kaznelson, as well as our own for Mr. V., indicate an osmotic pressure of the appetite gastric juice not far below or above that of the human blood. According to Bickel (13) the gastric juice (10-year-old child) is always hypotonic to the blood. Lehmann (15) concludes that the osmotic pressure of normal gastric juice (gastric content) is usually less than −0.50° C. and that a concentration above this figure indicates hyperacidity or other pathological conditions. This view is obviously untenable.

The osmotic concentration of the dog's appetite gastric juice appears to be practically identical with that of man. Sasaki (14) reports a variation from −0.51° to −0.60°; Rosemann gives somewhat higher figures as −0.56° C. to −0.64° C. On the other hand, Bickel (13) reports extraordinary fluctuations in osmotic concentrations of dogs gastric juice (Pawlow pouch) or −0.52° to −1.21° C. We question whether the normal stomach can secrete a juice of the osmotic concentration −1.21° C., that is, twice that of the blood.

IV. *The nitrogen of the gastric juice.* 1. The total nitrogen was determined by the method of Kjeldahl on nine different lots of appetite gastric juice of Mr. V., with the following results:

Appetite gastric juice	*Nitrogen per 100 cc. gr.*
Lot I	0.074
Lot II	0.051
Lot III	0.054
Lot IV	0.057
Lot V	0.054
Lot VI	0.051
Lot VII	0.075
Lot VIII	0.057
Lot IX	0.071
Average	0.060

The average of all our determinations is 0.060 gr. nitrogen per 100 cc. appetite juice. The total nitrogen of the hunger juice was not determined. If all the nitrogen is in the form of proteins, and if we accept the figures of Nencki and Sieber, and of Pekelharing (16) namely, that the nitrogen constitutes 14.39 per cent of the proteins of the gastric juice, the appetite gastric juice of man would contain on the average nearly 0.42 gr. protein per 100 cc. This is practically all the total organic solids in the appetite juice (Table I). It is therefore probable that some of the nitrogen in the gastric juice is present in non-protein combinations, such as ammonia, amino acids, and sulphocyanic acid.

The literature does not, to our knowledge, contain any data on the total nitrogen of pure human gastric juice. Rosemann reports nitrogen determinations on two lots of dog's appetite gastric juice, finding 0.035 gr. and 0.054 gr. per 100 cc. respectively. The reader will note that these figures are considerably lower than those on the appetite juice of Mr. V.

2. *The ammonia of the gastric juice.* The ammonia of the fresh gastric juice was determined by a combination of Folin's aeration and the Nessler colorimetric methods, using 1 to 5 cc. of the juice. It appears that the ammonia cannot be determined by the Nessler reagent directly in pure gastric juice, as parallel tests on the same samples of gastric juice yielded higher figures by aeration and Nessler than by Nessler direct (using 1 cc. of the juice).

Our data, summarized in Table III, go to show that

1. Ammonia in the amounts of 2–3 mgr. per 100 cc. is a constant constituent of pure gastric juice of man and dog.

2. The ammonia appears to be slightly more concentrated in the continuous secretion or hunger juice than in the appetite juice.

3. The ammonia appears to be greatly increased in gastric ulcers (dog).

Rosenheim (17) and Strauss (18) reported small amounts of ammonia in the gastric content of man. Zunz (19), working with the gastric content (test meals) on normal persons and on persons with various disorders of the alimentary tract, also reports the presence of ammonia. In the normal individuals the ammonia of the test meal contents varied from 0.7 to 5.0 mgr. per 100 cc. In cancers of the stomach the ammonia in the test meal content is greatly increased, according to Zunz's figures. Zunz concludes there is no relation between the state of the gastric digestion or the acidity of the gastric

content and the quantity of ammonia in the gastric content. Nevertheless, the test meal introduces factors (bacterial action, saliva, etc.) not present in pure gastric juice. Sommerfeld (13), working with pure gastric juice of a ten-year-old girl with complete stricture of the oesophagus, states that gastric juice contains no ammonia. Nencki, Pawlow, Zaleski (20) and Salaskin (21) reported 4 to 4.5 mgr. ammonia per 100 cc. pure gastric juice of the dog. Rosemann (2) reports

TABLE III

The ammonia in gastric juice

INDIVIDUAL	MATERIAL	NO. OF OBSERVATIONS	NH_3 IN MGR. PER 100 CC. GASTRIC JUICE		
			Maximum	Minimum	Average
Mr. V.	Appetite juice	30	5.5	2.0	3.0
	Hunger juice	10	5.6	3.0	4.0
Mr. E*	Appetite juice	8	3.0	1.5	2.0
Mr. C.†	Appetite juice	5	8.5	7.5	8.0
	Hunger juice	3	10.0	9.5	9.8
6 normal dogs with Pawlow pouches	Appetite juice	20	4.5	1.5	2.5
	Digestion juice	24	4.5	1.5	2.5
Dog‡ Pawlow pouch and ulcer in pouch	Continuous secretion	5	25.0	10.0	18.0

* Mr. E. is a second gastric fistula case, a man age 24, partial cicatricial stenosis of oesophagus from drinking lye. Gastrostomy of five months' standing. The man is in good general health, and shows no gastric disturbance.

† Mr. C. noticed increased hunger pains, and at times slight epigastric pain for a period of several months when these tests were made. There was no variation in the diet.

‡ This dog had an experimental ulcer in the stomach pouch produced by intravenous injection of a strain of streptococci isolated from a gastric ulcer in man by Dr. C. E. Rosenow. This dog was used in another line of work by my assistant, Mr. L. L. Hardt.

the constant presence of a small amount of ammonia in the pure gastric juice (appetite secretion) in the dog. Reisner (29) concludes that the ammonia in gastric juice comes from the saliva.

What is the origin and significance of the gastric juice ammonia? It is known that saliva contains traces of ammonia. We find that the mixed saliva of man (A. J. C.) contains from 0.5 to 1.5 mgr. ammonia per 100 cc. Salaskin found 2.5 mgr. NH_3 per 100 cc. in the saliva of the dog. But in dogs with Pawlow's stomach pouch, and in my gastric

fistula case, Mr. V., no saliva can enter the stomach or the part of the stomach yielding the juice. In the present work the saliva therefore is not a factor.

The ammonia of the duodenal content may be a factor, as Boldyreff has shown the frequency with which intestinal content enters the stomach. This factor is excluded in dogs with the Pawlow stomach pouch. In my human fistula case this factor is readily controlled by making the ammonia determinations only on those samples of gastric juice that are absolutely free from admixture with bile, pancreatic juice, and succus entericus.

Rosemann points out that the gastric juice ammonia cannot be a simple filtrate from the blood since normal blood contains only about 0.5 mgr. of ammonia per 100 cc.

The above considerations seem to limit the origin of the gastric juice ammonia to the following factors:

1. It may be an active excretion from the blood, in which case one would expect an increase in the gastric juice ammonia on increasing the blood ammonia. In normal individuals there appears to be a decrease in the elimination of ammonia by the kidneys during gastric secretion (Gammeltoft (28).

2. The ammonia may be formed in the secretion process itself.

3. The action of the hydrochloric acid on the proteins of the gastric juice (conversion of alkaline or neutral proteins to acid proteins).

4. The action of the hydrochloric acid on cells of the mucosa (splitting off of ammonia by the cells as a protective measure against the action of strong acids).

5. In the case of gastric ulcers of infectious origin ammonia may actually be produced continually by bacterial action in the active focus of the ulcer.

6. In the case of traumatic or non-infectious ulcers it seems highly probable that the action of the hydrochloric acid on the raw surfaces of the gastric mucosa will result in the formation of ammonium chloride.

In 1898 Nencki, Pawlow and Zaleski (20) reported studies on the ammonia concentration in the gastric mucosa and its relation to the secretion of gastric juice. They found that, per unit of mass, there is more ammonia in the gastric mucosa than in any other tissue of the body. 100 gr. gastric mucosa at rest contained 20 mgr. NH_3. 100 gr. gastric mucosa after 2 hours secretion (sham feeding) contained 42 mgr. NH_3.

These findings were essentially confirmed by Salaskin the same year. They seem to indicate some relation of the ammonia formation to the

secretion process itself, unless the higher ammonia content of the secreting mucosa represent ammonium chloride in the process of absorption from the gastric juice. The other possible factors referred to above are capable of experimenta tests and work is in progress in that line.

3. *The amino-acids of the gastric juice.* The formol titration was made on eight lots of appetite gastric juice of Mr. V., and on three lots of appetite gastric juice of Mr. E. When deductions were made for the ammonia nitrogen of the juice, the formol titrable nitrogen of Mr. V.'s gastric juice varied from 3 to 9 mgr. nitrogen per 100 cc. Mr. E.'s appetite juice gave 7 mgr. per 100 cc. Four lots of dog's appetite gastric juice (Pawlow pouch) gave only 1 to 2 mgr. of amino-acid nitrogen. It thus appears that normal human gastric juice contains slightly more amino acid than ammonia nitrogen; but the greater part of the gastric juice nitrogen is associated with the more complex proteins.

Zunz (19) working with gastric contents after test meals reports that the amino acid nitrogen usually exceeds the ammonia nitrogen, and that both substances are increased in cases of gastric cancer. In three normal persons the maximum amino acid nitrogen was 10 mgr. per 100 cc. of gastric content, while in several gastric cancer cases it reached 15–20 mgr. per 100 cc. of content. But these figures cannot be directly compared with ours on pure gastric juice, because of the uncertain factors associated with the gastric contents following a test meal.

4. *The auto-digestion of the gastric juice.* When fresh gastric juice is incubated at 38° C. the following changes take place in the proteins and the gastric mucin.

(1) All the ropy mucin and mucin flocculi are dissolved.

(2) The pink color of the biuret reaction is increased. In fact, fresh human gastric juice gives practically a violet biuret reaction, and this color is intensified and changed towards pink by the auto-digestion.

(3) The characteristic protein precipitation at the point of neutralization is decreased.

(4) The quantity of proteins precipitated by nitric acid and by heat is reduced.

It is thus clear that the proteins of pure gastric juice undergoes pepsin-hydrochloric acid digestion in the stomach itself. But some of the gastric juice proteins are not hydrolyzed, at least not down to the peptone stage. It has been pointed out in a previous report (1) that this auto-digestion of the gastric juice itself is probably a factor in the continuous secretion of gastric juice in the way of yielding gastric secretagogues.

TABLE IV

Anaphylactic reactions of guinea pigs, using human gastric juice and human serum as sensitizing and toxic agents. Pigs 7, 8 and 9 were sensitized with human gastric juice that had been incubated at 38°C. for 10 days

G. PIG	SENSITIZING DOSE	INTERVAL	TOXIC DOSE	ANAPHYLACTIC SYMPTOMS
1	0.1 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
2	0.2 cc. fresh g. juice	15 days	4 cc. fresh g. juice	Doubtful
3	0.5 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
4	1.0 cc. fresh g. juice	15 days	4 cc. fresh g. juice	Doubtful
5	1.5 cc. fresh g. juice	15 days	2 cc. fresh g. juice	Doubtful
6	2.0 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
7	2.0 cc. digested g. juice	20 days	1 cc. human serum	Severe; death in $1\frac{1}{4}$ hrs.
8	1.0 cc. digested g. juice	20 days	3 cc. fresh g. juice	None
9	0.2 cc. digested g. juice	20 days	3 cc. fresh g. juice	None
10	1.0 cc. fresh g. juice	23 days	0.5 fresh human serum	Severe; death in 1 hr.
11	2.0 cc. fresh g. juice	23 days	5.0 fresh g. juice	Doubtful
12	5.0 cc. fresh g. juice	23 days	5.0 fresh g. juice	Slight
13	0.5 cc. fresh g. juice	27 days	5.0 fresh g. juice	Doubtful
14	1.0 cc. fresh g. juice	27 days	0.5 cc. human serum	Severe; recovered
15	5.0 cc. fresh g. juice	27 days	5.0 fresh g. juice	None
16	0.5 cc. fresh g. juice	30 days	0.5 cc. human serum	Severe; recovered
17	1.0 cc. fresh g. juice	30 days	5.0 fresh g. juice	Doubtful
18	2.0 cc. fresh g. juice	30 days	5.0 fresh g. juice	None
19	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
20	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
21	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
22	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
23	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
24	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
25	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
26	5.0 cc. human serum	18 days	10.0 fresh g. juice	Doubtful
27	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
28	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
29	0.05 cc. human serum	27 days	1 cc. human serum	Severe; death in $1\frac{1}{2}$ hrs.
30	0.05 cc. human serum	27 days	10.0 fresh g. juice	No symptoms
31	0.10 cc. human serum	27 days	2.0 cc. human serum	Severe; recovered
32	0.10 cc. human serum	27 days	10.0 fresh g. juice	None
33	0.15 cc. human serum	27 days	2.0 cc. human serum	Severe; recovered
34	0.15 cc. human serum	27 days	10.0 fresh g. juice	Doubtful
35	0.25 cc. human serum	27 days	1.0 cc. human serum	Severe; death 1 hr.
36	0.25 cc. human serum	27 days	10.0 fresh g. juice	None
37	0.5 cc. human serum	27 days	1.0 cc. human serum	Severe; death 1 hr.
38	0.5 cc. human serum	27 days	10.0 fresh g. juice	None

5. *The nature of the gastric juice proteins and their relation to the serum proteins as shown by the anaphylactic reaction.*

This series of experiments on guinea pigs with human gastric juice as a sensitizing and toxic dose for anaphylaxis were made as a preliminary step for the study of pathological gastric juice. The injections were made intraperitoneally with a blunt needle. The gastric juice was rendered slightly alkaline by titration with $\frac{N}{10}$ NaOH. It is well known that at the neutral point of the gastric juice some of the proteins are precipitated, and that these go into solution on rendering the juice slightly alkaline. The human serum was obtained, not from Mr. V. who yielded the gastric juice, but from Mr. Rogers, who assisted in this part of the work.

The results are summarized in Table IV. These data seem to show that: (a) *Normal human gastric juice contains a sensitizing substance for human serum, but that it is practically devoid of toxic substances both in relation to the proteins of the gastric juice and to serum proteins.* This is rather an unexpected situation, in view of the fact that the gastric juice contains complex proteins in considerable amounts. (b) The gastric juice contains some practically unchanged serum proteins capable of acting as sensitizer, but incapable or in too small concentration to act toxically. This sensitizing substance is not diminished or destroyed by auto-digestion of the gastric juice at 38° C. for ten days (Table IV, pigs 7, 8, 9).

It is of interest in this connection to recall that according to Gay and Adler (22) the euglobulin (of horse serum) is sensitizing but not toxic. And Wells (23) has shown that enzyme (trypsin) hydrolysis of proteins destroys the toxic properties of the proteins very must faster than the sensitizing properties, the latter persisting even after three years continued tryptic digestion.

V. *The acidity of normal gastric juice.* The acidity was determined by titration with $\frac{N}{10}$ NaOH, and using dimethyl-amino-azo-benzene and phenolthalein as indicators for the free and the total acidity respectively. During the three years that Mr. V. has been under observation hundreds of determinations have been made of the acidity of the contents of the "empty" stomach, of the hunger juice or continuous secretion, and of pure appetite juice. The reader will recall that the contents of the "empty" stomach is taken one hour after washing out the stomach with 200 cc. of water. All the cases where the gastric juice or gastric content was contaminated with bile (intestinal content) are excluded from the summaries given in Table V.

The second gastric fistula case, Mr. E., is a man 26 years of age, healthy and vigorous. Nearly a year ago his oesophagus was corroded with a solution of lye, and this led to a nearly complete cicatricial stenosis. The gastrostomy was made in October, 1914. I am under obligations to Dr. Bayard Holmes for the privilege of working with Mr. E. daily for two weeks. My observations were made in February, 1915. At that time the oesophagus had been dilated sufficiently to permit swallowing of any well masticated food and the gastrostomy opening was used only in the dilation processes. In this case saliva is therefore not excluded from the contents of the empty stomach, and possibly not from the continuous or hunger secretion, although Mr. E. was instructed and urged not to swallow any saliva during these experiments. The appetite juice was obtained by Mr. E.

TABLE V

The acidity of normal human gastric juice

PERSON	MATERIAL	NO. OF OBSERVATIONS	ACIDITY					
			Free			Total		
			Low	High	Aver.	Low	High	Aver.
Mr. V.	Cont. empty stomach.	235	0.10	0.35	0.18	0.15	0.40	0.23
	Hunger juice.....	180	0.15	0.35	0.25	0.20	0.45	0.34
	Appetite juice.....	285	0.35	0.44	0.40	0.40	0.53	0.48
Mr. E.	Cont. empty stomach.	10	0.09	0.36	0.20	0.18	0.41	0.25
	Hunger juice.....	8	0.20	0.32	0.25	0.27	0.38	0.33
	Appetite juice.....	15	0.30	0.36	0.34	0.36	0.47	0.44

chewing palatable food, and spitting out the chewed food, care being taken not to swallow saliva or particles of food.

These results on my two gastric fistula cases are in agreement with the work of Pawlow and his pupils on dogs, and the work of previous observers on pure gastric juice of normal persons. The latter data have recently been brought together and discussed by Boldyreff (24).

Normal human gastric juice (appetite secretion) when secreted above a certain minimum rate shows a practically constant total acidity of nearly 0.5 per cent HCl, or the same as the gastric juice of normal dogs. The gastric juice (appetite as well as hunger juice) secreted by the normal stomach at a low rate shows lower than normal acidity and total chlorides. The view of Pawlow based on experiments on dogs that gastric juice is secreted at uniform and constant acidity is true for man only

in regard to the appetite, digestion, and hunger juice secreted at fairly high rate. We must take cognizance of the equally important fact that the normal gastric mucosa is capable of secreting a juice of submaximal acidity.

Last year Rehfus and Hawk (25) reported a series of interesting observations on the acidity of the gastric content at varying periods after drinking water, and ingesting an Ewald test meal, which they offer as "direct evidence of the secretion of gastric juice of a constant acidity in the human stomach," and as "the first direct demonstration in the human subject in favor of Pawlow's theory of the secretion of a juice of a constant acid concentration." Rehfus and Hawk are dealing with the gastric content, not with pure gastric juice. The fact that the gastric content soon reaches and then maintains for some time a fairly constant acidity indicates the equilibrium between the acid secretion and the factors of neutralization, as shown by Boldyreff, but it does not show that the juice is poured into the stomach at a certain constant acidity. Analysis of the Ewald meal or the continuous secretion do not permit us to draw any conclusion in regard to the acidity of the actual gastric juice. If the authors mean by the above conclusions that the gastric juice of normal persons, that is the pure appetite juice, or the juice secreted during the first two or three hours of digestion exhibit a fairly constant acidity of nearly 0.5 per cent HCl, they are undoubtedly correct; but this conclusion is not new, as shown by Boldyreff's review of the earlier literature, and it cannot be based on the observations they report.

The reader will note that *normal human gastric juice is equal in total acidity to the maximum acidity reported by clinical observers for so-called hyperacidity in man.* So far as I am acquainted with the literature, *there is no evidence that the gastric glands under any pathological conditions are able to or do secrete a juice of higher than normal acidity.* Moreover, the presence in the stomach of gastric juice of full acid strength leads by itself and immediately to no untoward symptoms.

The contents of the "empty" stomach, and the continuous or hunger secretion (when the secretion rate is low) have uniformly a lower acidity than the appetite juice. The total acidity of contents of the "empty" stomach is about 0.2 per cent. The reader will note that this figure is frequently given, especially by clinicians, as the acidity of pure gastric juice of normal persons. The acidity of the continuous or hunger secretion is higher, and the greater the secretion rate the higher the acidity until it may equal that of the appetite juice. In no instance

does the acidity of the continuous secretion exceed that of the appetite juice.

What is the cause of the low acidity of the continuous secretion and contents of the empty stomach? The following factors must be taken into account:

1. The actual acidity of the juice as secreted may increase with the secretion rate, until the maximum acidity is reached with the high average rate of secretion, a condition similar to that obtaining in the case of the salivary glands where the concentration of the salts and the organic materials increase with the rate of a salivary secretion. If this is a factor the gastric juice secreted at a low rate should show a lower osmotic concentration and total chlorides than the juice secreted at high rate. The figures reported by Umber (12) for man and by Rosemann (2) for the dog appear to support this view, the former investigator showing particularly that the osmotic concentration (Δ) of the gastric juice increases with the rate of secretion. The cryoscopic data may, however, be misleading, as the salts produced by the neutralization of the HCl may not dissociate as freely as the acid.

2. The slower rate of secretion may give chance for the HCl to be partly neutralized by the alkaline mucus secreted by the mucin cells of the gastric mucosa. This is the factor emphasized by Pawlow. In fact, Pawlow takes the position that in the normal animal gastric juice has practically a constant acidity, irrespective of the secretion rate, but the actual acidity of the juice in the cavity of the stomach is purely a matter of rate of neutralization. If this is the sole factor, the total chlorides of the gastric juice ought to show a greater constancy than the acidity. That the hydrochloric acid of the gastric juice is in part neutralized by the gastric mucus is obvious. But according to Boldyreff the alkalinity of gastric mucus is only 0.05–0.10 per cent Na_2CO_3. That is to say, it would require 50–100 cc. gastric mucus to reduce 100 cc. gastric juice from the normal acidity of 0.45 per cent down to 0.25 per cent. The importance of this factor has therefore been overestimated by Pawlow.

3. When the gastric juice is collected from a Pawlow accessory stomach, or from an individual with complete closure of the oesophagus, as is the case with Mr. V., the saliva cannot be a factor in lowering the gastric juice acidity by neutralization and dilution. When all or most of the saliva is swallowed the acidity of the gastric juice is necessarily reduced in proportion to the relative rate of salivary and gastric secretion. This is effected by dilution rather than by neutralization,

as the titration alkalinity of saliva is low (0.08 per cent Na_2CO_3, Neumeister, cited by Boldyreff).

4. According to Boldyreff the most important factor in lowering the acidity of gastric juice from that actually secreted by the gland (0.5 per cent) to that usually found in the cavity of the stomach (0.25 per cent) is the entrance of intestinal contents (pancreatic juice, bile, and succus entericus) into the stomach. Boldyreff has shown that this occurs in dogs, and probably occurs in man, when the acid in the stomach mounts much above 0.25 per cent. I am satisfied from my observations on Mr. V. that Boldyreff's view is essentially correct. When 15–25 cc. appetite gastric juice of full normal acidity is permitted to remain in the empty stomach, even a few minutes, the pyloric sphincter dilates, and the contents of the upper end of the duodenum is in some way forced into the stomach. The quantity of intestinal content entering the stomach at any one time varies from a trace up to 10 cc., and the acidity of the gastric juice is correspondingly reduced by dilution and neutralization. The presence of duodenal content in the empty stomach is therefore a normal occurrence, but the mechanism involved in this intestinal regurgitation has not been closely worked out. We know from the work of Cannon and others that an acid reaction on the stomach side of the pylorus tends to dilate the pyloric sphincter. But it is difficult to see how a patent pylorus can cause the pancreatic juice and bile to enter the stomach without an actual antiperistalsis in the duodenum.

It is clear that it is this "mechanism for self regulation of the acidity of the stomach content" (Boldyreff) which breaks down in cases of so-called "hyperacidity" in man. In cases of "hypersecretion" the quantity of juice secreted is greater than normal, and the secretion may persist in the absence of all normal stimuli, but the neutralizing factors suffice to reduce the acidity of the juice approximately to that found in the normal stomach. It is purely a balance of secretion rate and of neutralization capacity. Impairment of the neutralization factors or very excessive secretion rate of gastric juice would tend to render the acidity of the gastric content equal to that of pure gastric juice; in other words, produce clinical "hyperacidity."

VI. *The total chlorides of the gastric juice.* The total chlorides of the gastric juice were determined by the method of Volhard on 10 different lots of appetite juice and 11 different lots of the hunger juice or continuous secretion. The results are summarized in Table VI. The secretion rate and the acidity of the various lots of juice are also

recorded. It will be seen that the total chlorides of the appetite juice are very constant, the minimum being 0.49 per cent and the maximum 0.56 per cent chlorine. The continuous secretion or hunger juice is more variable in chloride content, and this variation appears to be directly dependent on the secretion rate and on the acidity. In general the lower the secretion rate the lower the acidity and the lower

TABLE VI

The total chlorides of normal human gastric juice

NO. OF EXP.	GASTRIC JUICE	SECRETION RATE PER HR.	ACIDITY %		TOTAL CL. %
			Free	Total	
		cc.			
1.....	Hunger juice	6	0.20	0.32	0.40
	Appetite juice	560	0.42	0.48	0.56
2.....	Hunger juice	4	0.18	0.25	0.25
	Appetite juice	308	0.41	0.48	0.53
3.....	Hunger juice	8	0.30	0.40	0.38
	Appetite juice	500	0.41	0.49	0.54
4.....	Hunger juice	12	0.30	0.41	0.44
	Appetite juice	398	0.42	0.49	0.55
5.....	Hunger juice I	2	0.20	0.32	0.28
	Appetite juice	154	0.42	0.48	0.56
	Hunger juice II	10	0.29	0.38	0.36
6.....	Hunger juice	2	0.20	0.33	0.34
	Appetite juice	200	0.41	0.46	0.53
7.....	Hunger juice	6	0.30	0.41	0.43
	Appetite juice	280	0.45	0.52	0.56
8.....	Hunger juice.....	3	0.18	0.28	0.27
	Appetite juice	150	0.41	0.47	0.53
9.....	Hunger juice	2	0.14	0.29	0.24
	Appetite juice	210	0.43	0.50	0.56
10.....	Hunger juice	3	0.26	0.36	0.36
	Appetite juice.....	100	0.32	0.40	0.49
11.....	Hunger juice	36	0.34	0.40	0.53
	Appetite juice (mixed with bile)	228	0.32	0.37	0.56

the total chlorides. This is in agreement with the findings of Foster and Lambert (26) on dogs.

These facts seem to point to the conclusion that the low acidity of the gastric juice secreted at a slow rate is not due entirely to neutralization by alkaline mucus. We have apparently a secretion of gastric juice of an acidity actually lower than that of the rapidly secreted

appetite juice. The dependence of the actual secreted acidity on the secretion rate is not a very close one, however, as we may have very marked fluctuation in rate without any change in chlorides. But below a certain secretion rate (25–30 cc. per hour from the entire stomach of the adult) an actual hypoacid juice is secreted.

The above figures for total chlorides in normal human gastric juice agree closely with the findings of previous observers on the gastric juice of dog and of man. Rosemann gives 0.54–0.64 per cent Cl for the appetite gastric juice of the dog. The figures given by Sommerfeld for human appetite juice varies from 0.53 to 0.59 per cent Cl. Umber working on an old man (59 years) with partial esophageal stenosis (malignant) reports total chlorides of the gastric juice as varying from 0.27 to 0.60 per cent Cl. The reader will note that the lowest figure reported by Umber is practically identical with the lowest total chlorides found by me in the very slowly secreted hunger juice. It is probable that the low chloride gastric juice samples of Umber were secreted at a slow rate.

VII. *The concentration of pepsin.* Most of the pepsin determinations were made by the method of Mett; a few tests were also made with the ricin, and the U. S. Pharmacopœia methods. The tubes (2–2½ mm. diameter) were sealed at both ends with heat and the egg albumin coagulated by boiling for 10 minutes, and the tube left in the water until cooled, and then set aside for two days before using.

The digestion mixture was made up as follows: 1 cc. gastric juice + 15 cc. $\frac{N}{10}$ HCl, + two Mett's tubes (1½ cm. length) at 37° C. for 24 hours. The $\frac{N}{10}$ HCl was used because it approximates the free acidity of appetite gastric juice, although we are aware of the fact that this degree of acidity is somewhat higher than the optimum for artificially prepared pepsin (cf. Cobb (27), Sörensen (30), Michaelis and Davidsohn (31)). We propose to determine the optimum acidity for the pepsin in normal gastric juice in a later work. It would seem singular if normal gastric juice is secreted with an acidity too great for optimum peptic digestion. It may be so, however, for under normal conditions the actual peptic digestion must be carried out under less than normal acidity of the gastric juice, because of dilution with saliva and the water of the food, and the fixation of the acid by the protein of the food.

Our results with the Mett's test on the gastric juice of Mr. V. may be given in the following summary:

Appetite gastric juice.
Number of tests (Lots of G. J.): 45.
Digestion in mm.: High, 8½; low, 6; *average, 7.*
Hunger juice (continuous secretion):
Number of tests: 35.
Digestion in mm.: High, 8½; low, 5; *average, 6.*
Contents of the "empty" stomach:
Number of tests: 35
Digestion in mm.: High, 4; low, 3; *average, 3½.*

The results are stated in the length of albumin column actually digested, because according to Cobb the law of Schütz does not hold for pepsin in concentrations that digest more than 4–5 mm. in 24 hours.

The variations in pepsin concentrations in these three groups of

TABLE VII

The relation of total acidity and pepsin concentration (Mett's method) in normal human gastric juice (Mr. V).

SERIES	CONTENTS "EMPTY" STOMACH		HUNGER G. JUICE		APPETITE G. JUICE	
	Acidity	Pepsin	Acidity	Pepsin	Acidity	Pepsin
	per cent	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*
18	0.18	4	0.40	6	0.48	7
28	0.10	3	0.25	6	0.48	7½
35	0.18	3½	0.41	6½	0.47	7
38	0.15	4	0.38	7	0.48	7½
45	0.15	4	0.40	6½	0.49	7½
95	0.22	4	0.28	6½	0.41	7
100	0.10	3	0.35	6	0.44	6½

gastric juice, and the absence of a close relation of pepsin concentration and acidity is further illustrated by a few typical series of tests given in greater detail in Table VII.

The appetite gastric juice of Mr. E., our second gastric fistula case, when tested as above in 14 experiments showed a pepsin concentration of 5–7 mm. with an average of 6 mm., a slightly lower value than the gastric juice of Mr. V.

We have tested as above the gastric juice (appetite, as well as digestion secretion) of 25 dogs with Pawlow fistula. The dogs, normal and in good condition, were being used in other lines of work. We were surprised to learn that the dog's gastric juice showed uniformly a lower pepsin concentration than the gastric juice of man. The figures ob-

tained on dog juice was: highest, 5 mm.; lowest, 2 mm., with an average of $3\frac{1}{2}$ mm. The reader will note that this is only about half the quantity of digestion obtained in the human juice.

When the Mett's tubes are placed in 16 cc. of undiluted human gastric juice (appetite secretion) the digestion in 24 hours at 37° C. varies from 12–16 mm., or only twice the quantity digested in the dilution of 1 cc. juice to 15 cc. $\frac{N}{10}$ HCl. This seems to indicate that *in normal gastric juice the pepsin is present in excess of the needs or at least far in excess of that needed in economic digestion.*

The U. S. Pharmacopœia defines "100 per cent Pepsin as a preparation capable of digesting three thousand times its own weight of finely divided egg white (coagulated) in three hours." The Pharmacopœia test is carried out as follows: 10 grams of boiled white of egg is macerated through a No. 40 filter and placed in 40 cc. 0.3 per cent HCl, $3\frac{1}{3}$ mgr. dried pepsin added, and the mixture incubated at 52° C. for three hours, with occasional stirring When thus carried out there is only a very small residue of undissolved egg white at the end of three hours, but the procedure to measure the amount of this residue does not yield very accurate results.

This test was applied to six different lots of appetite gastric juice of Mr. V. Under above conditions, 1–$1\frac{1}{2}$ cc. appetite gastric juice digest 10 gr. of coagulated and finely divided egg white in three hours practically as completely as is done by $3\frac{1}{3}$ mgr. "100 per cent pepsin." As defined by the U. S. Pharmacopœia, 1 cc. of human gastric juice must therefore contain $3\frac{1}{2}$ mgr. pepsin, or 100 cc. of the juice, 35 mgr. pepsin. We have seen that the appetite gastric juice of man contains about 400 mgr. organic material per 100 cc. That is, according to the Pharmacopœia definition, only about 10 per cent of the organic matter in the human gastric juice is pepsin. Of course, we are aware of the fact that the Pharmacopœia definition and test is only one of convenience for standardizing commercial preparations of pepsin, and having no direct relation to pepsin concentration in normal gastric juice.

It has been shown in a previous report that an adult normal person if hungry secretes 600–700 cc. gastric juice on an average palatable dinner, or about 1500 cc. gastric juice total in 24 hours. That is to say, there is a secretion of 240–250 mgr. pepsin per dinner, capable under proper conditions of digesting from 630 gr. to 750 gr. of protein (coagulated and finely divided egg albumen) in three hours; and the total pepsin secretion in 24 hours is 525 mgr., capable of digesting $1\frac{1}{2}$ kilo proteins (coagulated egg white) in three hours.

The practically complete digestion of 10 gr. boiled and finely divided egg white by 1 cc. human gastric juice in three hours is a fact. The secretion of 600–700 cc. gastric juice on an avergage dinner by the adult human stomach is an estimate, but not far from the actual fact. It is therefore clear that the normal human stomach secretes pepsin far in excess of the *actual needs* of gastric digestion, or, more precisely, far in excess of what can be used advantageously under ordinary conditions of gastric digestion When the boiled egg white is broken up in larger pieces, such as occurs in ordinary rapid mastication, 1 cc. of gastric juice requires 6–10 hours for complete digestion.

This great excess of pepsin in normal gastric juice probably explains the clinical findings of great reduction in pepsin content without any evidence of impaired gastric digestion, provided sufficient acid is present. It probably also explains, in part at least, the practical uselessness of commercial pepsin as a therapeutic measure in gastric disorders.

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THE CONTENT OF SUGAR IN THE BLOOD OF CATS UNDER THE INFLUENCE OF COCAINE

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It has become apparent that the chemical composition of the blood is very rapidly changed by the conditions to which animals are subjected both preliminary to and during the operation of drawing samples of blood. Especially is this true with reference to the carbohydrates.

Thus Pavy (1) shows not only that it is necessary that the animals be kept free from excitement while the sample is being drawn, but also that when the animal is killed and all the blood is used, ". . . it is necessary to proceed with the utmost expedition in order that no change may take place in the contents of the circulation from the *post mortem* production of sugar in the liver." These results have been substantiated by many investigators since the publication of Pavy's work and are treated in considerable detail in a recent paper by Scott (2), in which the results of extensive researches are given, with numerous references to the literature both on glycaemia and glycosuria. Still more recently Shaffer (3) shows that the sugar content of the blood of the dog when "free from excitement or pain is surprisingly low and constant." Scott also discusses the effects of chloroform and ether, and records a few results from the use of cocaine.

It is the purpose of this paper to put the effects of cocaine to a more thorough analysis by means of a larger series of experiments. The chemical technique used in the experiments here recorded is the same as that described by Scott in the paper above referred to, and need not be repeated. The work was performed under the immediate direction of Dr. Scott, and it is a pleasure to acknowledge my indebtedness to him.

DESCRIPTION OF EXPERIMENTS

In these experiments rather large doses of cocaine hydrochloride dissolved in M/8 sodium chloride solution were given. It is obvious

that if results to which any reliability can be attached are to be expected, certain standard conditions must be determined upon which the effect of the drug itself can be superimposed. The conditions adopted in this work are the same as those reported by Scott in the paper above referred to, pages 278 to 293. The chief essentials are that the animals were kept in captivity for from ten to twenty days under excellent conditions and were fed exclusively on meat, the natural diet of the cat.

It is well known that certain other processes of metabolism are affected by cocaine injections; therefore it was necessary to proceed with extreme caution in the carrying out of the experiments, as well as in their interpretation. For example, Araki (4) found that lactic acid was eliminated in the urine of frogs and rabbits after cocaine injections; also one rabbit out of four secreted sugar with the urine. Underhill and Black (5) report that while small doses of cocaine, 10 mgm. per kilo of body weight, cause no appreciable influence upon the course of nitrogenous metabolism or the utilization of protein and fat, larger doses, 15 to 20 mgm. impair, first, fat utilization and, subsequently, that of protein, while a marked decline in body weight is manifest. It has also been shown by Scott and others that the character of the diet upon which animals are fed will affect the sugar concentration. Besides this it is quite evident from the work of a number of investigators that the glycosuria which has been found in many cases, as well as the hyperglycaemia which is known to exist even before any sugar is excreted, is frequently due to the emotional condition of the animal entirely apart from the diet or the anesthetic.

For these and other reasons only such animals as conformed to the standard conditions were used in these experiments. Throughout the work great care was taken to avoid everything that would in any way tend to excite the animals before drawing the blood for analysis. It was of course impossible to do this with absolute success in every case, inasmuch as some of the cats which it was found necessary to use were unaccustomed to being handled, even though not particularly wild. Others were somewhat wild. When, however, anything occurred that might indicate other causes than that of the drug for hyperglycaemia strict records of such conditions were made. The animals were killed by sudden decapitation, and the blood was obtained from the severed vessels of the neck.

In table 1 are given the results from five animals which were almost entirely quiet throughout the handling preliminary to the injection

and during the operation itself. It will be noticed that the amounts of sugar in the blood here recorded are very close together, the highest variation from the mean being only 15 per cent. In fact the variation is but little greater than that found by Scott in normal cats, or than is to be expected from unavoidable variations in the internal relationships with their necessary readjustments, as is pointed out by Cannon (6).

TABLE 1

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	VAR. FROM MEAN	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*		
1	F	18	2.80	0.070	6	62.83	0.0736	+11	Fairly quiet
2	F	18	3.30	0.070	6	79.85	0.0720	+ 9	Quiet
3	F	12	3.00	0.050	10	77.38	0.0679	+ 2	Ideal
4	F	14	2.20	0.050	10	50.11	0.0619	− 7	Practically quiet
5	F	17	1.90	0.050	8	49.90	0.0546	−15	Slightly excited before, but quiet during injection
Mean							0.0664		

The results from the four excited animals reported in table 2 would scarcely be expected to vary greatly from those of table 1, since the excitement, while evident, was not at all excessive. The mean is somewhat higher than the mean of table 1, which result is in accord with the results obtained by various investigators already referred to.

TABLE 2

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	VAR. FROM MEAN	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*		
6	F	18	2.6	0.045	11	81.45	0.0815	+ 9	Excited from first Cried during injection
7	F	12	2.3	0.050	5	58.63	0.0643	−14	Cried during injection
8	M	14	2.8	0.050	5	80.00	0.0832	+11	Restive
9	M	17	2.15	0.050	6	64.65	0.0695	− 7	Restless; slightly excited
Mean							0.0746		

This conclusion is strengthened by the interesting examples of the two individuals recorded in table 3. These animals showed exceptional reaction to the drug. In the first there was no particular excitement manifest before the injection, but as soon as the operation was over marked excitement was noticed.

Protocol of Experiment 10

Time of injection 10.05. Quantity, 27 mgm. per kilo of body weight.
10.08. Cries, due to drug; had not been excited.
10.09. Evidence of nausea; deep respiration; vomiting.
10.10. Pupil reflex slow.
10.12. Hind legs weak; respiration rapid; cries; salivation.
10.15. Spasms; after about 10 seconds the first spasm diminished slightly, and the animal was killed just as the second spasm set in; blood seemed cyanotic.

TABLE 3

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*	
10	F	11	2.6	0.070	10	54.52	0.1393	Quiet at start; drug showed marked influence; nausea and spasms.
11	F	11	2.5	0.035	27	82.92	0.2215	Cat fought during injection

In the other experiment, No. 11, the cat was difficult to handle and fought from the first. The injection was not entirely successful, only about one-half of the syringeful of cocaine being introduced. Since the cat was rather large the injection was thought hardly sufficient to yield adequate results, hence the animal was rejected and returned to the cage. In a short time, however, the characteristic symptoms were manifest: intense salivation, dilated pupils, etc. The cat was killed twenty-seven minutes after the injection. It will be noticed that the result in No. 10 was higher than in any other except No. 11, yet the quantity of cocaine used in the former was approximately the same as that in other cases; in the latter case the quantity used was only about half as great, yet the carbohydrate recovered is extremely high. The reason why the result in No. 11 is higher than in No. 10 is possibly twofold. In the first place the excitement manifested by the cat was very much greater, and, secondly, the time between the injection and the killing was longer. This, however, merely substantiates the results of a number of investigators previously reported.

CONCLUSIONS

1. If we compare the amount of sugar in the blood of cocainized cats in table 1, mean 0.0664, with Scott's standard of 0.069 for normal animals, it is seen that the mean of the series here presented is slightly lower than the standard. This might be taken to indicate that the direct influence of the cocaine tends to diminish the concentration of the carbohydrate in the blood, and is thus in harmony with the short series reported by Scott. This difference, however, is so slight that for most purposes it may be neglected.

2. While an increased concentration of sugar was noted in the blood of several cocainized animals it seems obvious that this was due to excitement. It seems a matter of indifference whether this excitement is due to the action of the drug or to other causes. In this connection it may be noted that while the cocaine may truly cause hyperglycaemia it does so only in an indirect manner.

3. While cocaine may be used for local anesthesia, in drawing blood for sugar analyses its use involves an element of doubt that would better be obviated if possible.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXVI. The Relation between the Digestion Contractions of the Filled, and the Hunger Contractions of the "Empty" Stomach

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Boldyreff in 1905 (1) first reported that there occurred contractions of the empty stomach of the dog during starvation. Cannon and Washburne (2) reported that the same occurred in man. Carlson (3) by more delicate methods, analyzed the various types of motor activities of the empty stomach during prolonged hunger. His work showed that the empty stomach exhibits three types of activity.

1. Rhythmical contractions occurring about three times per minute designated the "twenty second rhythm."

2. Very vigorous contractions occurring periodically. The duration of each of these contractions is about thirty seconds.

3. Tonus changes of the stomach musculature.

In his first paper Carlson states that probably the twenty second rhythm is the peristaltic activity of the antrum pylori. The thirty second rhythm or hunger contractions were thought to be contractions of the fundus. His later work on dogs with Pawlow pouches showed that the twenty second rhythm is not confined to the pylorus, but is also exhibited by the fundus (4). The general trend of all of Carlson's experiments is toward the conclusion that the first period of hunger contractions apparent after a meal is an exaggeration of the motor activities of the stomach occurring during gastric digestion.

In experiments on the rabbit (5) undertaken at Dr. Carlson's suggestion it was found difficult to differentiate the hunger contractions of the stomach from the digestion peristalsis. No other conclusion seemed possible than that the hunger contractions were augmented peristaltic contractions.

In conference with Dr. Carlson the following experiments were outlined to determine whether or not the same conclusion applied to man and dog:

We used the two standard methods of investigating the movements of the stomach: the rubber balloon and the X-ray methods. To study the genesis of the hunger contractions the rubber balloon, which was connected by small size rubber tubing with the recording manometer, was swallowed shortly after a meal and continuous graphic records of the intragrastric pressure variations were made until after the onset of a typical hunger period.

Soon after eating an average meal the subject of the experiment swallowed the balloon and put himself into a comfortable position either sitting in a chair or lying on a cot. The results were similar, irrespective of the position; the best results were obtained while the subject was asleep for then inhibiting psychic influences were removed. During a long continued series of tracings we tried to keep the balloon in the upper part of the stomach by pulling it up at intervals and swallowing it again. We realize it is possible for it to be pushed to the pyloric end of the stomach, but by the X-ray observation in the dog we found that the balloon introduced after feeding remained in the cardiac end of the stomach for a considerable time.

In order to actually see the hunger movements we coated a balloon with a bismuth paste and observed its movements in the stomach with the X-ray.

The only practicable way we found of preparing these balloons consisted in painting the outer wall of one balloon with a bismuth paste prepared by mixing bismuth subnitrate with vaseline. This balloon was then inclosed within another of the same size; thus the two balloons were separated by a thin wall of bismuth paste. These balloons had to be prepared anew for each experiment, as the peristaltic waves tend to sweep all the bismuth to the lower end of the balloon and the vaseline soon causes the rubber to lose its elasticity. We were able to make graphic tracings with simultaneous direct fluoroscopic observation.

Our records show that the fundus is quiescent immediately after eating a meal. The pressure upon the balloon is maintained at a steady level. If a light meal is taken the tonus variations may be demonstrated immediately after eating. At first they are so slight as to seem insignificant but they increase in vigor and are usually visible thirty minutes after the meal. In one experiment an unusually large meal was eaten and the tonus waves were distinctly in evidence twenty

minutes later (fig. 1). They increase in intensity and may but do not always, become more rapid. Each wave is of one to three minutes' duration. When the stomach is nearly empty (as determined by the stomach tube or induced vomiting) they become conspicuous and at this stage of digestion there usually appear superposed upon them, stronger contractions which increase in vigor and are felt by the subject as hunger pains (fig. 2). Although it is by no means always the

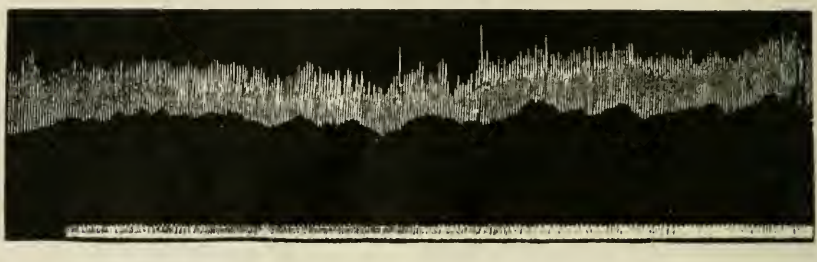

Fig. 1. Tonus rhythm of fundus of stomach (man) 20 minutes after a hearty dinner. Time in seconds.

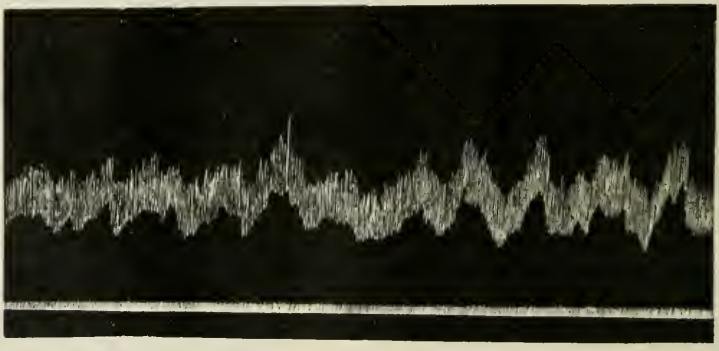

Fig. 2. Tonus rhythm of stomach (man) three hours after dinner (beefsteak, spaghetti, bread, butter, apples and cream).

case, it is significant that the first contractions felt may occur when the stomach still contains traces of food.

Carlson (6) in his first paper describing the hunger contraction noted that the onset of a hunger period was marked by the appearance of a slow tonus rhythm which gradually increased in vigor and culminated in the hunger contractions. As stated above this tonus rhythm is

present not only as an immediate precursor of the hunger period but also throughout the course of normal gastric digestion.

Many workers have employed the rubber balloon in recording the stomach movements but none of the published tracings so far as we are aware, indicate the presence during normal digestion of a slow continuous rhythm as herein described.

Moritz (7) reported contractions of the fundic end of the stomach occurring two and one-half to three and one-half times per minute. According to Dietlan (8) the time required for the peristaltic contractions of the pyloric end of the stomach is twenty to twenty-four seconds. We, therefore, think as suggested by Cannon and Carlson that the tracings of Moritz record the intragastric variations due to the pyloric peristalsis.

Sick (9) using the same method reported that there occur tonus variations of the fundic end of the stomach which give rise to the peristaltic waves. These occur at the rate of two to four per minute in the full or empty stomach. The tracings of Sick show three kinds of pressure variations: Respiratory, cardiac, and "Magen tonus schwangungen," the latter averaging twenty seconds each. The duration of these "stomach tonus variations" coincide with the time intervals required for the pyloric peristalsis.

Is the hunger contraction simply an augmented peristaltic contraction or a contraction of the fundus as a whole? To answer this we studied the stomach movements in the dogs and in ourselves by the "balloon X-ray" method. We were able to make direct observations of the movements of the balloon in the stomach and at the same time note the character of the graphic record.

The upper part of the balloon was held in the cardiac end of the stomach. A young and vigorous dog was employed for the experiments. The dog was starved for intervals of thirty-six to forty-eight hours and in order not to have the hunger contractions completely inhibited by the excitement attendant upon the X-ray examination, frequent repetitions were made until the dog became accustomed to the necessary manipulations. The vigor of the stomach contractions thus made visible to the naked eye is surprising. The weaker type of hunger contractions begin as a constriction in the cardiac end of the stomach and pass down toward the pyloric end as a rapid peristaltic wave. In the very vigorous contraction the wave spreads over the stomach so rapidly that it is difficult to decide whether there is a contraction of the fundus as a whole or a very rapid peristalsis (fig. 3).

Such a condition may well be compared to the peristaltic rush of the intestine as described by Meltzer and Auer (10).

When the X-ray observation of the hunger contractions were made in ourselves, with subject in reclining position a series of photographs were taken of the balloon at times when the record showed the stomach to be comparatively at rest and at varying intervals during a period

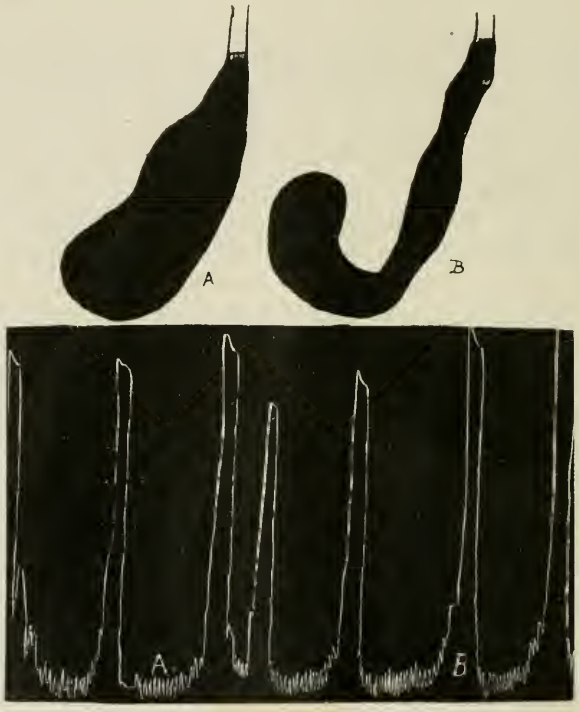

Fig. 3. Hunger contractions of dog's stomach after 30 hours' starvation. *A*, Outline of stomach (as seen by X-ray and bismuth balloon), between hunger contractions with stomach relaxed. *B*, Outline of stomach at the height of a hunger contraction.

of hunger contraction (figs. 4, 5, 6, 7). We made sure the balloon was just below the cardia by pulling the distended balloon upward as far as it would come. By careful observation we were able to tell from the movements of the balloon when a hunger pain was being felt by the subject. In every case the graphic record and the subject

confirmed our own observations. When a hunger pang was felt, a series of constrictions passed rapidly over the balloon. Beginning at the cardiac end, they swept rapidly toward the pyloric end increasing in strength as they proceeded. It was readily seen that the hunger contractions are powerful peristaltic contractions, which, arising at or near the cardiac sphincter, swept downward over the entire stomach. During a typical hunger period the stomach exhibits movements which resemble very closely the movements which have been described by clinicians in patients after a bismuth meal as hyperperistalsis, but described by Cole as normal peristalsis of a stomach that contains small quantities of food. Observations of the hunger contractions of the dog were made after thirty-six hours' deprivation of food, on ourselves after fifteen hours. Prolonged starvation in the case of the dog was necessary to overcome the inhibitory influence of the excitement attendant upon the X-ray examination. Whether or not the stomach of man after a longer period of starvation would show a condition approaching that described for the dog, we are not in a position to state.

The current teaching with reference to the part played by the fundus during digestion is that it is a reservoir, exerting a tonic grasp upon its contents. The kinematographic figures of the stomach published by Kästle, Ridder, and Rosenthal show that the fundus is not quiescent during digestion. Cole (12) has shown that when food is in contact with the cardiac end of the stomach, contractions begin in the fundus and frequently are as deep in this region as in the pylorus. Forsell (13) describes the fundus changes as follows:

> In the standing position, the fundus shows no peristalsis. In the reclining position there occur typical persitalses of the fornix. The fornix empties itself principally by a concentric contraction of the whole wall. At the conclusion of gastric digestion there occur circular contractions of the wall. One may then observe stages in which the fornix assumes a more oval form: then a circular contraction presses the food into the korpus: then a stage of rest in which the fornix takes a more spherical shape. The peristaltic period is begun by a transverse division of the fornix which presses the fornix into a more cylindrical shape and forces the contents into the korpus. This is repeated at intervals until the fornix is empty.

These circular contractions of the stomach may be the cause of the rhythmical pressure variations which we found and which we here speak of as tonus rhythm. That there is a certain degree of independence between the slow tonus rhythm and the hunger contractions is shown by the behavior toward inhibiting agents. Carlson (14) showed

Fig. 4. X-ray photograph (Dr. Rowntree), of the bismuth balloon in the stomach when the graphic record shows the stomach to be quiescent.

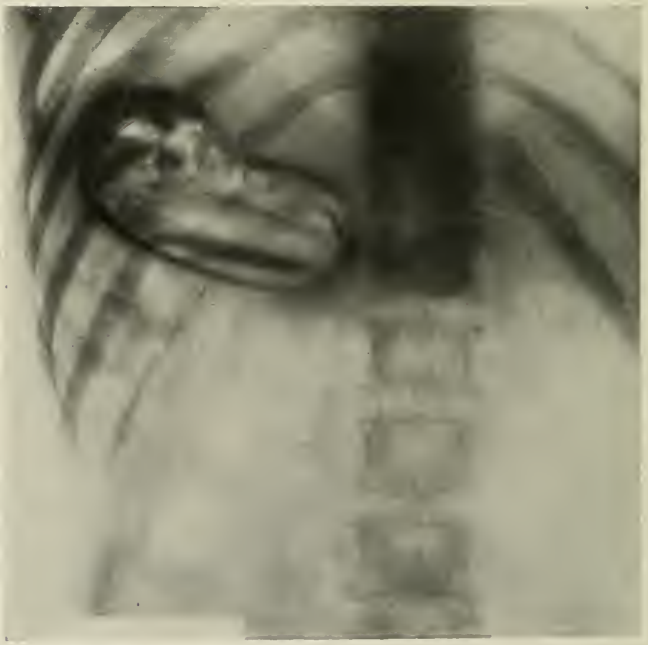

Fig. 5. X-ray photograph (Dr. Rowntree), of the balloon in the stomach at the height of a moderately strong hunger contraction.

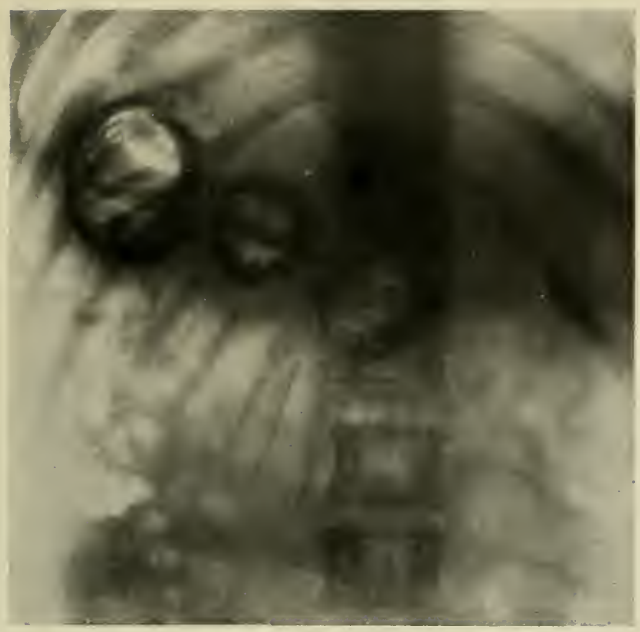

Fig. 6. X-ray photograph (Dr. Rowntree), of the balloon in the stomach at the height of a very strong hunger contraction.

Fig. 7. X-ray photograph (Dr. Rowntree) of the balloon in the stomach near the end of a hunger contraction.

that numerous substances and circumstances inhibit reflexly the hunger contractions. This we found true in ourselves, but this tonus rhythm does not show the same sensitiveness toward the same inhibiting influences. Thus tasting sugar or acid, swallowing water or 0.36 per cent hydrochloric acid solution in small quantities (10—25 cc.) do not inhibit this rhythm or only very slightly (fig. 8). This is in contrast to the immediate effect of the same substances on the hunger contractions.

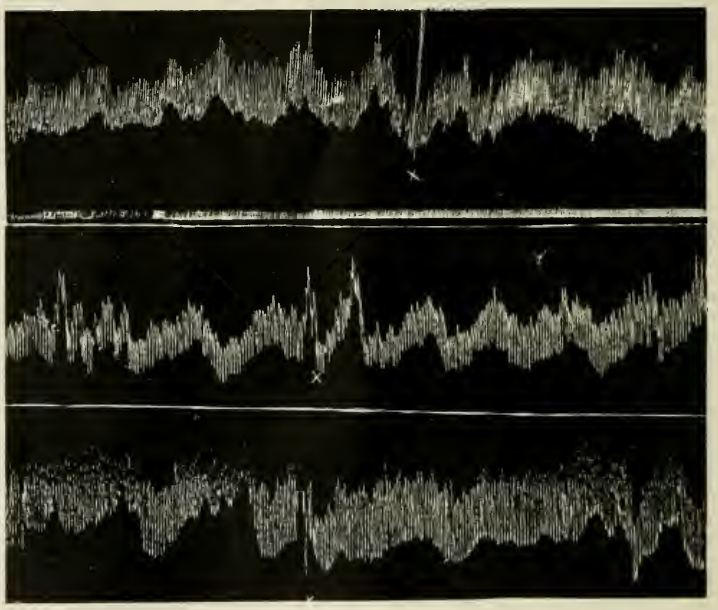

Fig. 8. Tonus rhythm of stomach (man) during gastric digestion is not inhibited to the same degree by substances which completely inhibit the hunger contractions. *A*, at *x* 25 cc. of 0.36 per cent HCl was swallowed. *B*, at *x* 50 cc. of cold water swallowed. *C*, Tonus rhythm just before a hunger period; at *x* 50 cc. of cold water was drunk.

Large quantities of liquids do inhibit the tonus rhythm probably as a result of the distension which a sudden increase in volume of gastric contents would necessitate. A quantity of liquid which does not inhibit the rhythm in a filled stomach may do so in an empty stomach. Excitement, interest, worry or any strong psychic influences immediately inhibit the rhythm. The twenty-second rhythm is not so easily inhibited as either the hunger contractions or the slower tonus rhythm.

Our findings interpreted in the light of the contributions of Cannon, Carlson and Forsell show that the normal stomach exhibits the following types of muscular activities:

1. A tonic grasp of the upper stomach musculature upon the food. This tonic condition exhibits slow rhythmical variations.
2. Peristaltic contractions of the antrum pylori.
3. Peristaltic contractions of the entire stomach (the hunger contractions of Boldyreff, Cannon and Washburne, and the thirty-second rhythm of Carlson).
4. In the young dog the vigorous hunger contraction begins with a constriction at the cardiac end and is followed by a contraction of the entire fundus. (If this type of contraction be peristaltic the waves are too rapid to be detected by our methods.)

During normal digestion the peristaltic waves sweep over the lower part of the stomach. The fundus exhibits a rhythm of the same rate as was shown by the work of Carlson and Orr. In the meantime there are slow rhythmical tonus variations of the upper part of the stomach. As the stomach empties itself the peristaltic waves arise from points higher and higher toward the cardiac end of the stomach run over the entire stomach, culminating in a more or less tetanic contractions of the antrum. The hunger contractions are therefore strong peristaltic contractions in a stomach whose condition compared with its normally filled condition is hypertonic. Evidently these two phases of stomach contractions are most intimately related. The tonus may become so great as to run into tetanus or it may maintain a level on which the separate peristalses are superposed. These two different activities are not necessarily similarly affected by the same inhibiting influences. Both may be inhibited by introducing food or liquid into the mouth or stomach or by psychic influences but not to the same degree.

CONCLUSION

1. Both in man and in the dog, the fundic end of the stomach, during normal digestion, exhibits a slow tonus rhythm of one to three minutes' duration. During the emptying of the stomach and the onset of the hunger period, this tonus rhythm becomes more vigorous.
2. In the dog after thirty-six hours' starvation the stomach is markedly hypertonic; a vigorous hunger contraction is a contraction of the fundus as a whole.
3. In man after fifteen hours' starvation the hunger contractions

are vigorous peristaltic contractions in groups of 2–4, superposed upon the slow tonus rhythm. They begin at the cardiac end of the stomach and sweep over the whole stomach.

4. A given inhibiting agent does not necessarily have the same effect upon the different types of gastric contractions; thus the pyloric peristalsis is the most resistant to reflex inhibiting influences; fundic peristalsis is least resistant, while the tonus rhythm occupies a position between the two.

We wish to express our thanks to Dr. Rowntree of the Presbyterian Hospital under whose supervision the X-ray work on ourselves was carried out, and to Dr. A. J. Carlson for his help and criticism.

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A CONTRIBUTION TO THE PHYSIOLOGY OF LACTATION

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FOREWORD

The development and functioning of the mammary gland in the female mammal is closely associated with, and dependent upon the reproductive function.[1] The normal course of activity of the gland is somewhat as follows.

Embryonic— The first sign of the mammary organs, the milk line, appears at an early stage in embryonic life, and growth is continued until the gland reaches a considerable development at

Birth— At birth a milky fluid may be expressed from the gland in either sex, indicating a secretory activity, but this activity soon ceases and the gland remains quiescent until

Puberty— At puberty under the influence of the ovaries growth is resumed and carried to a greater or less development, varying with the individual and the occurrence of

Pregnancy— Pregnancy induces a great hypertrophy of the gland, and it reaches a high state of development, accompanied by the accumulation of colostrum, by the time of

Delivery— At this time milk secretion proceeds actively and if the milk is removed at short intervals, by nursing or artificially, secretion continues for a period of days, months, or years. The rate of secretion, however, after a time gradually decreases to zero. This decrease is favored by a succeeding

Pregnancy— Further growth of the gland ensues but not as marked as in the preceding gestation, and at

Delivery— milk secretion is again actively resumed. Whence, the pregnancy-delivery cycle is repeated.

[1] The mammary gland may, in turn, influence the reproductive and other body functions, although such influence is, apparently, not so fundamental.

This paper presents some data and discussion based on an investigation of certain phases of the above outlined course of activity of the mammary gland carried out at the Hull Physiological Laboratory of the University of Chicago. I wish to acknowledge especially the assistance and suggestions of Dr. Carlson throughout the work. I am also indebted to Dr. Mathews and Dr. Koch, among others, for various suggestions.

EXPERIMENTAL METHODS AND RESULTS

Prenatal development. The milk lines may be distinguished as early as the 4 mm. stage in the human embryo, according to Strahl (1) Knoepfelmacher (2) states that in children born prematurely there is no appearance of milk in the mammary glands. In a post mortem examination of two kids, the mother of which died about two weeks before term, I found mammary tissue plainly developed but no appearance of milk in the transected gland. Chemical examination for the presence of sugar was not made and this test might have shown the presence of milk in amount too small to be distinguished by simple cutting of the gland. The udder of the mother, a multipara, was at the time undergoing a rapid hypertrophy as evidenced by increase in size. (Data on this point will be found in table 1, Goat No. 4). A part of the increase in size was due to the accumulation of colostrum, which exuded freely from the cut ducts of the gland after death.

The influence of pregnancy on the growth of the mammary gland. The growth of the mammary gland during gestation is a very striking phenomenon. This development is in preparation for its function of milk secretion following delivery. Ribbert (3) transplanted the mammary gland of a guinea pig into the skin back of the ear. Five months later (the gestation period is about two months) living young were born, whereupon the transplanted gland functioned promptly. Ribbert states that hypertrophy was not sufficiently marked to make the gland apparent beneath the skin, that the nipple did not develop, and there was, therefore, no outlet for the secretion, but that milk was present in the gland. Marshall (4) cites the case of the Bohemian pygopagous twins, Rosa-Josepha, one of whom became pregnant. The breasts of both underwent development during pregnancy and functioned following delivery. Lane-Claypon and Starling (5) produced a development of the mammary glands in a virgin rabbit closely resembling that occurring during pregnancy by means of injecting extracts of rabbit foetus.

On the other hand, Lombroso and Bolaffio (6) using parabiotic methods with rats found no evidence that the pregnancy of one of a pair of females produced any effect on the mammary glands of the other one of the pair. Knoepfelmacher (2) injected blood serum from a pregnant goat into a non-lactating goat. The amounts used were 55, 33, and 90 cc. taken respectively 6 days, 2 days, preceding delivery, and 1 day following delivery. The results were negative.

I have injected defibrinated blood from a pregnant non-lactating, multiparous goat into a non-pregnant, non-lactating, multiparous goat with negative results with reference to increase in the volume of the mammary glands or milk secretion. The volume of the udder was determined by pressing a suitable vessel containing warm water up

TABLE 1

Showing the effect on the volume of the udder of a non-pregnant goat from the intravenous and intraperitoneal injection of defibrinated blood from a pregnant goat

GOAT NO. 4—DONOR (PREGNANT, NON-LACTATING, MULTIPAROUS)		GOAT NO. 3—RECIPIENT (NON-PREGNANT, NON-LACTATING, MULTIPAROUS)		
Date	Volume of udder	Volume of udder	Blood injected	Method of injection
1915	*cc.*	*cc.*	*cc.*	
1–21	935	170	90	Intraperitoneal
1–23	935	165	115	Intraperitoneal
1-25	950	175	100	Intraperitoneal
1–27	980	175	75	Intraperitoneal
1–30	1005	160	15	Intravenous
2-3	1060	170	85	Intraperitoneal
2–6	1090	170	35	Intraperitoneal

around the udder and firmly against the body wall. The amount of water displaced (determined by the difference between the amount left in the vessel and its capacity) was taken to represent the volume of the glands. The method is not exact but is sufficiently accurate to show any marked changes in volume. The data are given in table 1. No appearance of milk resulted during the period of injections or following.

The figures in table 1 show that the udder of the donor was increasing steadily in size but the amount of blood injected failed to affect the size of the udder of the recipient. The latter at a previous time during a similar stage of pregnancy as represented by the donor in the present instance had fully as large an udder as the donor. The figures, then

serve to show the enormous difference in size of the udder with the stage of pregnancy.

The influence of pregnancy on milk secretion. Lane-Claypon and Starling (5) advance the theory in connection with the work referred

TABLE 2

Daily milk and fat yield of goat No. 2, showing the effect of intravenous injections of defibrinated blood from a pregnant goat, No. 3; from her two days old kid; and again from No. 3 six days after delivery. No. 2 said to have dropped her kids about April 12. No. 3 kidded August 8, 1914

DATE	MILK	FAT	FAT	REMARKS
1914	*cc.*	*per cent*	*gms.*	
7–24	823	5.1	41.9	
7–25	796	6.4	51.0	
7–26	730	6.4	46.8	
7–27	645	5.4	35.0	
7–28	720	6.4	46.3	Injected intravenously 175 cc. defibrinated blood
7–29	**463**	**6.9**	**31.9**	from No. 3 (10 days before term)
7–30	708	6.8	48.2	
7–31	674	6.2	41.7	
8–1	783	6.8	53.7	Injected intravenously 300 cc. defibrinated blood
8–2	**552**	**7.0**	**38.6**	from No. 3 (7 days before term)
8–3	783	7.0	54.8	
8–4	847	7.8	66.1	Injected intravenously 175 cc. defibrinated blood
8–5	913	7.5	68.4	from No. 3 (3 days before term). Caused some
8–6	**349**	**4.9**	**17.1**	general depression, weather hot, flies trouble-
8–7	633	8.0	50.8	some
8–8	832	6.2	51.6	
8–9	752	5.5	41.1	
8–10	665	5.9	39.3	Injected intravenously 100 cc. defibrinated blood
8–11	**538**	**6.6**	**35.4**	from male kid of No. 3. Age of kid 2 days
8–12	806	6.8	54.7	
8–13	873	6.0	52.4	
8–14	853	6.5	55.0	Injected intravenously 100 cc. defibrinated blood
8–15	**765**	**7.0**	**53.7**	from No. 3 (6 days after delivery)
8–16	880	6.3	55.4	
8–17	823	7.3	59.6	
8–18	848	6.2	52.3	

to above that the developing foetus passes a substance into the maternal circulation which favors the growth of the mammary gland but inhibits its secretory activity. The removal of this inhibitor, then, at delivery allows the gland to function in its milk secretory capacity.

Bearing on this point I have made the following series of injections

of defibrinated blood into a lactating goat: 1, from a pregnant goat near term; 2, from a kid of this same goat 2 days after birth; 3, from the same goat 6 days after delivery. Data of this test are given in table 2.

The figures indicate an inhibitory action from each of the transfusions. The milk and fat data as given for each day are the sum of two milkings made throughout at approximately 12-hour intervals. In every case the inhibitory influence was most marked at the first milking following the transfusion. Except in the case of the transfusion made August 5 there was no apparent systemic disturbance

TABLE 3

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-freed cow's placenta; also, defibrinated blood from goat No. 5. No. 6 said to have kidded March 12, 1915. No. 5 dropped kids April 17, 1915, and at the time of transfusion, April 24, was milking around 2000 cc. per day

DATE	MILK	FAT	FAT	REMARKS
1915	*cc.*	*per cent*	*gms.*	
4–18	475	6.8	32.3	
4–19	488	7.3	35.5	
4–20	460	6.4	29.3	Injected intraperitoneally a water extract of 5 gms. of dried, fat-freed cow's placenta
4–21	**168**	**9.4**	**15.8**	
4–22	238	8.5	20.4	
4–23	365	6.7	24.4	
4–24	315	5.9	18.5	Injected intraperitoneally 200 cc. defibrinated blood from No. 5 (fresh 7 days and giving about 2000 cc. daily)
4–25	**182**	**8.6**	**15.7**	
4–26	**147**	**7.1.**	**10.5**	
4–27	**111**	**9.4**	**10.4**	
4–28	183	7.8	14.3	
4–29	154	8.3	12.8	

in the condition of the recipient. Leaving this case out of consideration the inhibitory action seems to be equally marked for either the blood of the pregnant goat or that of her two-day old kid.

The transfusion made 6 days after delivery had only a very slight inhibitory action as seen in the yield of milk and was not at all reflected in the yield of fat. Results in another trial with two other goats were similar to the above except in the transfusion following delivery (see table 3).

Effect of placental extract on milk secretion. Niklas (7) found that an extract of placenta caused milk production in virgins and mothers.

Lederer and Pribram (8) found greatly increased secretion of milk upon intravenous injection of placental extract.

Table 3 presents the data of a test of the effect of an extract of cow's placenta on milk secretion in the goat. The fresh placental tissue was ground, dried, extracted with gasoline and ether to rid of fat, and the residue extracted with water as needed. The figures show a distinct inhibition, quite similar in nature to that just observed from the blood of a pregnant goat or new-born kid (see table 2).

Relation of the blood to milk secretion. The activity of the mammary gland with reference to milk secretion varies enormously. A large part of the time it may be at zero, but immediately following a normal pregnancy and delivery it is at a high point. To test for the presence of a substance in the circulating blood stream which accelerates the secretory function of the mammary gland during the period of its highest activity following delivery, transfusions were made from a fresh, heavy milking goat to one giving a low yield. No increase in secretion was obtained by this means. In table 2 no change, or a very slight decrease was shown. In table 3 there is shown a very marked decrease. In this latter case the donor was milking heavily at the rate of about 2000 cc. daily, including the milk taken by two nursing kids. The recipient although giving a relatively low yield, about 300 cc. daily, had been fresh only about 6 weeks and would seem to have offered a favorable subject for positive results. No general disturbance was apparent to account for the decrease noted.

The effect of mammary gland extract on milk secretion. Gavin (9) fed mammary gland preparations to cows without affecting the yield or quality of the milk. The mammary gland of the same cow whose placenta was used in the data shown in table, 3, was prepared in the same way as given for the placenta. Results from the injection of water extracts of this material are given in table 4.

The figures appear to show a temporary inhibition followed by an increase to something above the previous yield. The net result of the several injections seems to be a considerable increase, but it may be that this should not be attributed to the injected material. Reference to table 3 shows that goat No. 6 was, a few days before, giving more than the yields recorded in table 4, and the apparent increase may be nothing more than recovery following the blood transfusion of April 24. However, the immediate inhibition, similar to that noted for the placental extract, seems to be unequivocal.

The action of pituitrin on the mammary gland. Since Ott and Scott (10) first noted that pituitrin had a galactagogue action this material has received considerable attention in its relation to milk secretion. All reports of investigators on the immediate action of pituitrin confirm the observation of Ott and Scott, viz., that its injection into the circulation causes an immediate flow of milk in a lactating animal. MacKenzie (11) working with cats in anesthesia and observing the flow of milk from the cut surface of the gland found pituitrin the most active of the several animal extracts he studied. Gavin (9) working with

TABLE 4

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-freed cow's mammary gland (pregnant). No. 6 said to have dropped kids March 12, 1915

DATE	MILK	FAT	FAT	REMARKS
1915	*cc.*	*per cent*	*gms.*	
5–3	113	8.3	9.4	
5–4	85	6.7	5.7	
5–5	90	7.7	6.9	
5–6	119	7.6	9.0	Injected intraperitoneally water extract of 5 gms.
5–7	**75**	**6.5**	**4.9**	cow's mammary gland
5–8	135	6.2	8.4	
5–9	143	6.6	9.5	
5–10	176	6.4	11.2	Injected intraperitoneally water extract of 1 gm.
5–11	**157**	**7.1**	**11.1**	cow's mammary gland
5–12	190	6.9	13.1	Injected intraperitoneally water extract of 4 gms.
5–13	**134**	**7.8**	**10.4**	cow's mammary gland
5–14	189	7.4	13.9	
5–15	220	7.5	16.4	Injected intraperitoneally water extract of 5 gms.
5–16	**177**	**7.7**	**13.7**	cow's mammary gland
5–17	194	7.3	14.3	

cows yielding 15–35 pounds of milk daily found no effect on quantity or quality of milk as the average result of 3–5 days treatment with pituitrin. In some cases, he reports, there was a distension of teat and cistern with milk immediately following injection. Hill and Simpson (12) report similar results. Hammond (13) has studied in some detail the effect of pituitrin on the composition of milk secreted under its influence. He finds that the fat content is greatly increased and that the protein, sugar and ash content remain very constant. This refers to the milk secured immediately after the injection of pituitrin and is not in conflict with Gavin's results which referred to longer periods;

for the yield of milk and its fat content are later depressed. Hammond concludes from his data that the action of pituitrin is not muscular. Hill and Simpson (14) confirm Hammond's results as to the composition of milk obtained after the use of pituitrin except they did not find the depression in fat content later, as noted by Hammond.

Heaney (15) found in the human a contraction of the breast upon injection of pituitrin followed by a return to initial volume, and attributes the milk secretory action of pituitrin to its muscular effect.

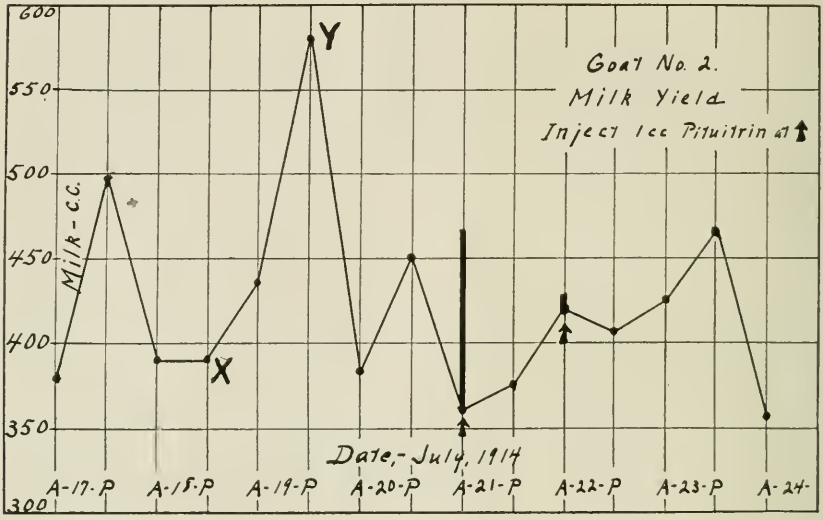

Fig. 1. Showing fluctuation in yield of milk by a goat milked at regular 12-hour intervals and the dependence of the flow of milk produced by the injection of pituitrin upon this fluctuation. The heavy vertical lines represent the yield of milk obtained upon intravenous injection of 1 cc. of pituitrin immediately after the regular milking. It is inferred, for example, that pituitrin would have caused a large further flow at X; while at Y little or no extra milk would have been obtained. A = morning milking; P = evening milking.

As to the quantity of milk and fat yielded as a result of the use of pituitrin (Parke & Davis preparation was used throughout the work here reported on pituitrin) based on the average of two days and not the immediate yield, my data show no effect either way. My results, based on three goats, confirm those of Hammond who found a decrease in fat content of the milk for a day or two following the use of pituitrin. I have found no marked or consistent variations in the sugar or protein content of the milk.

The flow of milk from an active mammary gland, which has not been drained for a few hours, upon injection of pituitrin is a very striking reaction. In the goat I have never failed to secure some flow of milk from its use even immediately after milking "dry." The amount obtained, however, varies considerably.

Figure 1 illustrates this point. The goat was milked regularly at 12-hour intervals but the yield is seen to fluctuate widely. The intravenous injection of pituitrin immediately following a regular milking produces a further flow varying in amount inversely with the yield secured by the hand milking which preceded it. That is, injection of pituitrin following a relatively high yield of milk produces a small flow; and following a relatively low yield it produces a large flow. A second, third or fourth dose produces only a very slight flow, 2–4 cc. in the goat.

I have followed the yield of milk in the nursing dog very satisfactorily by keeping the pups separated from the mother and allowing them to nurse at 8-hour intervals. The pups were balanced on a smooth-working balance, with shot, then allowed to nurse, and again balanced with the standard weights. The increase in weight represents approximately the yield of milk by the mother.

Under the above conditions, after the pups have nursed fully (6 or 7 minutes), I have never succeeded in producing any further flow of milk by the intravenous injection of pituitrin. But if the mother be placed under ether at the regular nursing hour and the pups then allowed to nurse the yield of milk is greatly depressed, although the pups do their part of the act in the usual vigorous manner. Often, in fact usually, the yield is so low as not to be detectable by the method used (that is, it appears, well under 5 gms., considering errors in loss of saliva, etc.). If, now, pituitrin be injected intravenously, with the mother still under ether, the pups immediately secure the normal yield, or the balance of it which they had failed to get in the first nursing, and this, in rather less time than is required in nursing under normal conditions. A second dose following the first in 10 minutes, produces no further flow.

Figure 2 shows an illustration of the effect of pituitrin under normal conditions and also with the mother under ether, but in the latter case some milk yielded to the pups nevertheless. Ordinarily the ether curve would hold to the base line until the pituitrin was administered.

While pituitrin causes no further flow of milk in the dog after nursing, this does not seem to hold true for the goat. Data from two

experiments are given in table 5 which show that after the kid had nursed and secured all the milk it could, injection of pituitrin still caused a further marked flow. The high fat content (15.8 to 19.6 per cent) of the milk thus obtained is also remarkable.

Bearing on the nature of the mechanism involved in the flow of milk produced by pituitrin under certain conditions, the following test was carried out. A cannula was inserted in the teat of a goat and connected with a chloroform manometer recording on the revolving drum of a kymograph. The gland was then inflated with air through a T-

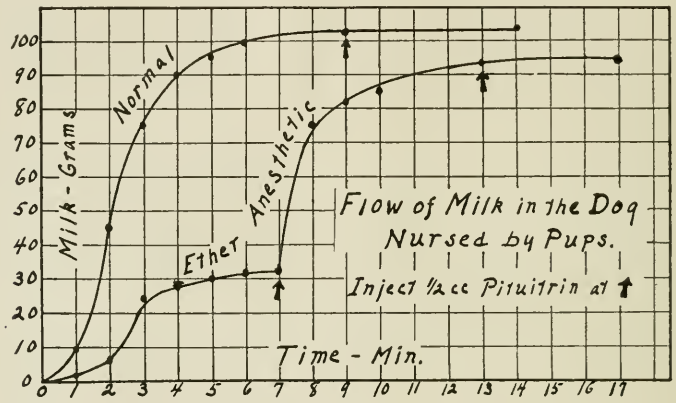

Fig. 2. Showing failure of pituitrin to cause a flow of milk following normal nursing in the dog; and, its restoral of the flow normal to the stimulus of nursing when the normal flow is inhibited by anesthesia; and, the failure of a second dose to cause any further flow. Also, showing under the stimulus of normal nursing the latent period in the flow of milk during the first minute, followed by the very rapid flow for the next two minutes, and then the gradual decline to zero (cf. figs. 4–8).

connection to a pressure of 8–10 cm. chloroform. The device may be made quite sensitive in recording any change in pressure within the gland. With the apparatus properly adjusted pituitrin was injected intravenously. The effect varies with the stage of activity of the gland. In the non-pregnant, non-lactating goat there is no apparent effect. This is true, also, in the pregnant, non-lactating goat up to a time close to term. A short time before term the result, following a latent period of 15–20 seconds, is a slight increase in pressure with a return to the initial. In the freely lactating goat, after a latent period

of 15–20 seconds, there is an abrupt rise in pressure followed by a gradual and nearly uniform decline to the initial pressure. The decline occupies about 5 minutes. A series of 3 curves, taken from the same goat before and after delivery, is given in figure 3.

A second dose of pituitrin in the freely lactating goat gives a curve similar to the first dose, except that it is much reduced in the extent of the rise. In the case of a goat milking from one gland and dry in the other, each gland being separately connected as above, pituitrin

TABLE 5

Showing the effect in the goat of nursing following milking "dry" by hand; and of pituitrin following nursing. After milking by hand, kid nursed left gland and at the same time right gland was milked by hand. Following the nursing 1 cc. pituitrin was injected intravenously and both glands milked by hand. Goat No. 5, kidded April 17, 1915

EXPLANATION	RIGHT GLAND			LEFT GLAND			BOTH GLANDS		
	Milk	Fat	Fat	Milk	Fat	Fat	Milk	Fat	Fat
	cc.	*per cent*	*gms.*	*cc.*	*per cent*	*gms.*	*cc.*	*per cent*	*gms.*
4–22–15									
Milked "dry" by hand							358	3.5	12.5
Nursed by kid				153			343		
Milked by hand	**190**	**9.8**	**18.6**						
Injected pituitrin—milked by hand	70	15.8	11.1	98	17.1	16.8	**168**	**16.6**	**27.9**
5–2–15									
Milked "dry" by hand							315	4.7	14.8
Nursed by kid				175			330		
Milked by hand	**155**	**13.3**	**20.6**						
Injected pituitrin—milked by hand	45	19.1	8.6	40	19.6	7.8	**85**	**19.3**	**16.4**

produced the typical curve in each gland, that is, the abrupt rise and slow decline in the active gland, and no change in the non-lactating gland.

While the above test indicates a contraction of the ducts and alveoli of the gland with a subsequent relaxation, it does not definitely localize the seat of the contraction. That is, the effect might be produced by the activity of the muscles of the large ducts. Indirect evidence on the point is afforded by the following test.

A lactating guinea pig was separated from her nurslings for two hours to allow the accumulation of milk in the glands. The animal was then anesthetised and a mammary gland dissected entirely away from the body with as little injury to the gland as possible. The end of the nipple was cut off to allow free egress to any milk. A few drops of pituitrin were now injected into the gland substance, whereupon there was a copious flow of milk from the cut nipple. A control injection of salt solution gave no such effect.

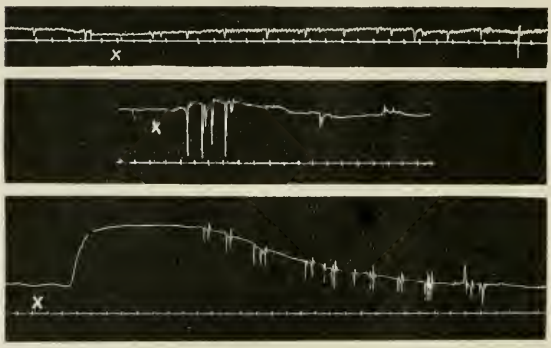

Fig. 3. Showing the effect of pituitrin on the pressure of an air-inflated mammary gland of a goat. The teat was connected with a cannula and tube to a chloroform manometer and the gland inflated to a pressure of 8–10 cm. At X $\frac{3}{4}$ cc. of pituitrin was injected intravenously. The time line marks 10-second intervals. The upper tracing was taken 37 days before delivery; the middle tracing, 17 days before delivery; the lower tracing, 12 days after delivery (the daily milk yield at that time being around 1800 cc.). The shallow waves, seen especially in the upper tracing, are due to respiratory movements. The deep, sharp irregularities are due to bleating and struggling of the animal.

A similar gland preparation was cut across with a razor, exposing a cut surface as remote as possible from the large ducts and vessels. A drop of pituitrin was placed on the cut surface and in a few seconds many minute white dots appeared beneath the pituitrin and slowly swelled to tiny milky rivulets streaming beautifully through the clear liquid. Similar preparations from the cat and dog gave like results.

The reflex involved in milking and nursing. According to Flower and Lydekker (16) the mother whale ejects the milk from her mammary glands into the mouth of the young without the active sucking on the part of the young which is generally common to mammals. Schäfer (17) states that the discharge of milk during the act of sucking or milking is in part the result of direct mechanical pressure upon the milk reservoirs of the larger ducts; and partly due to a contraction of the plain muscular tissue which accompanies these ducts, and which appears to

be set in action by mechanical stimulation of the nipple. He adds that the flow is probably aided by the swelling of the gland due to a reflex dilatation of its arterioles.

The curve of normal nursing in the dog given in figure 2 shows roughly the rate at which the pups secured milk during the process of nursing. The data on which the curve is based were obtained by working the pups in two relays of three pups each. Group I was allowed to nurse for 1 minute, then removed and Group II given way for 1 minute, Group I being meanwhile weighed. Places were then again exchanged and so on until they had obtained all the milk possible.

The normal curve shows that the pups could secure only a small amount of milk the first minute, but during the second and third minutes there was a relatively large amount of milk available to their

Fig. 4. Showing the pressure changes produced in one gland under the influence of kid nursing the other gland. Goat No. 5, fresh 10 days, milked 3 hours previously. Left gland connected with chloroform manometer (no inflation of gland). Kid nursing right gland. The sharp irregularities in the curve are due to bunting of the kid. Time line marks 10-second intervals and represents 7 cm. pressure (cf. figs. 5, 6, 7, 8).

efforts. After the third minute the amount available declines and at the end of 6 minutes the total supply appears to be exhausted. The very marked changes in the amount of milk secured with the progress of nursing are not attributable to any differences in the efforts of the pups themselves to secure their food.

In table 5 it is shown that, after milking the mother goat as completely as possible by hand, if a kid be allowed to nurse one gland he not only obtains a considerable quantity of milk but there is also a practically equal flow in the other gland which may now be readily obtained by hand milking. The full significance of this reaction with reference to the operation of a reflex contraction and ejection of milk from the gland is perhaps better given by the records of the following tests.

I. Change in pressure. One gland was connected by a cannula and tube to a chloroform manometer recording on a kymograph. A kid

was then allowed to nurse the other gland. An example of the effect is given in figure 4. The pressure registered upon insertion of the cannula was about 9 cm. During the period when the kid was nursing the curve shows a latent period of 60 seconds with no change in pressure aside from the purely mechanical effect of the impatient bunting of the kid. At the end of this latent period there appears a marked rise in pressure. That, coincident in time with this rise in pressure, the kid was obtaining a satisfactory supply of milk, was plainly evident from the cessation of his bunting (shown, as well, in the smoothness of the curve), his rapid guzzling, and the rounding out of his little paunch. Two minutes later a second kid was allowed to nurse and a rise in pressure occurred; again only after a latent period of about 1 minute.

II. Flow of milk. A cannula was inserted in one teat and the milk flowing through it led by a short tube to one limb of a U-tube of large bore. The U-tube was held in a vertical position and its other limb was provided with a float and rod recording on a kymograph after the usual fashion of a manometer. Enough water was put in the U-tube to allow the float to ride freely, so that the addition of any further liquid would be properly registered. The connection of the tube from the teat with the U-tube was open so that the liquid in both limbs was constantly under atmospheric pressure only. The outlet for the milk remained constantly about 10 cm. below the level of the teat. With the apparatus adjusted a kid was allowed to nurse the free teat. Examples of the results are given in figures 5–7.

In the experiment represented by figure 5 the right gland was connected as described above and the left gland milked by hand. After the hand milking was completed a kid was allowed to nurse the left teat. During the first 35 seconds of the kid's nursing the curve shows a flow amounting to a few drops only from the right gland. At the end of a 35-second latent period it shows a rush of milk reaching a maximum in 20 seconds and being practically complete in 80 seconds. The form of this curve is strikingly similar to that of the curve of flow in normal nursing of the dog shown in figure 2.

The high fat content (13.3 per cent) of the milk obtained under the stimulus of the nursing of the kid is noteworthy and significant. The amount of fat contained in the second portion was 50 per cent greater than that obtained under the stimulus of the first milking operation.

In the trial shown in figure 6 preliminary hand milking was not used. Fifteen cc. of milk flowed upon insertion of the cannula in the course of 3 minutes. Upon nursing of the opposite gland by the kid there is

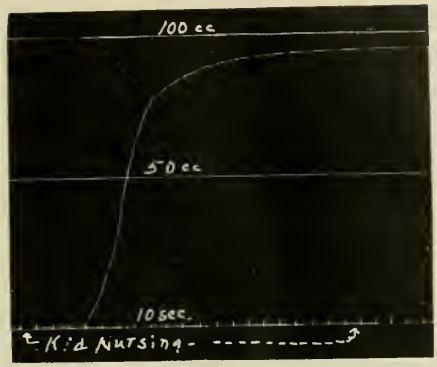

Fig. 5. Showing flow of milk through cannula from one gland under the stimulus of kid nursing the other gland. Goat No. 5, fresh 24 days, milked 11 hours previously. Cannula inserted in right teat and led to instrument recording the flow of milk. 84 cc. of milk flowed immediately on insertion of cannula. Left gland then milked as dry as possible by hand, yielding 136 cc. 45 seconds after the commencement of hand milking a further flow started from the right gland, yielding in 2 min. 68 cc. (results to this point not shown in the figure). Kid then nursed left gland causing a rapid flow from the right gland, after a latent period of 35 seconds as shown (cf. figs. 2, 4, 6, 7, 8).

		Milk, cc.	Fat, per cent	Fat, gms.
I	Left gland, hand milked	136	5.6	7.6
	Right gland, cannula flow	152	5.3	8.1
II	Left gland, kid nursed	90		
	Right gland, cannula flow	95	13.3	12.6

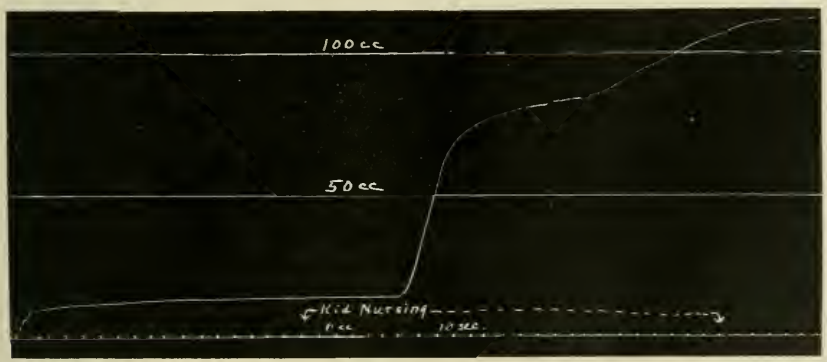

Fig. 6. Showing same as figure 5. Goat No. 5, fresh 11 days, milked $3\frac{1}{2}$ hours previously. Left gland connected with flow recorder, kid nursed right gland (no hand milking). Initial flow on insertion of cannula, 15 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 100 cc. Total flow 115 cc., 7.1 per cent fat (cf. figs. 2, 4, 5, 7, 8).

shown a latent period of 60 seconds and then a rapid flow from the unnursed gland. A second marked increase in flow is shown to occur 2 minutes following the first ejection. The curve shows, in comparison with figure 5, first, a longer latent period, and second, the occurrence of a second ejection of milk. In some cases the curve takes the form of 4 or 5 distinct steps.

The conditions of the test shown in figure 7 are practically identical with those of figure 6. The curve is given as showing another type of reaction, viz., a protracted and more or less uniform flow of milk (although a tendency to steps in the curve is shown here, also) under the stimulus of the kid's nursing. That the kid was also experiencing delay in the speed with which he was served his meal is evidenced by the length of time he continued to nurse.

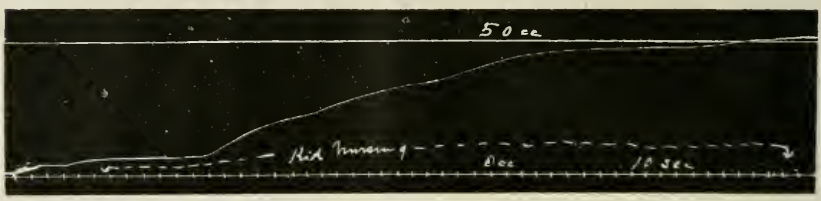

Fig. 7. Showing same as figure 5. Goat No. 5, fresh 16 days, milked 4 hours previously. Left gland connected with flow recorder. Kid nursed right gland (no hand milking). Initial flow on insertion of cannula, 7 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 44 cc. (cf. figs. 2, 4, 5, 6, 8).

Figure 8 brings out two points: (1) The curve of milk flow with the simple insertion of a cannula and without any other manipulation may take a distinct step form, as shown in the left hand part of the figure. (2) Hand milking of one gland produces a flow in the opposite gland, similar to the effect noted for the kid's nursing. (Other trials gave higher and steeper curves, very like that of figure 6.) The latent period is distinct, being 55 seconds in the present instance.

The question arises as to the nature of the factor or factors involved that cause the further ejection of milk upon nursing of the kid following hand milking, and which it seems to be impossible to call into play by usual methods of milking. I have tried various methods of electrical stimulation without success. I have imitated the nursing action

of the kid as actively as I could with my own mouth for several minutes at a time without result in the way of a further milk flow. The presence of the kid, nosing about the udder but not allowed to take a teat into his mouth, while using the greatest possible persuasiveness in the art of dry-hand milking was not sufficient. Neither did it suffice to add to the last mentioned recourse conditions of moisture and temperature by immersing the teat in water at 38–40°C. Only by allowing the kid to nose about the udder, while applying my own mouth to a teat in

Fig. 8. Showing flow of milk through cannula from one gland under stimulus of hand milking of other gland. Goat No. 5, fresh 23 days, milked 12 hours previously. Left gland connected with flow recorder. Initial flow on insertion of cannula, 27 cc., followed by second flow of 44 cc. Flow under influence of hand milking other gland, after latent period of 55 seconds, 33 cc. Total flow 104 cc. Kid then nursed right gland obtaining 140 cc. and causing a further flow from left gland of 124 cc. (not shown in the figure), (cf. figs. 2, 4, 5, 6, 7).

		Milk *cc.*	Fat *per cent*	Fat *gms.*
I	Right gland, hand milked	136	3.6	4.9
	Left gland, cannula flow	104	3.3	3.4
II	Right gland, kid nursed	140		
	Left gland, cannula flow	124	9.3	11.5

imitation of his sucking, have I been able to bring about the desired reaction without the actual nursing of the kid. The following protocol illustrates.

Goat No. 5. 5/16/15, 7.15–7.45 p.m.

	Milk *cc.*	Fat *per cent*	Fat *gms.*
Milked by hand in usual manner (kid not around)	285	7.9	22.5
Sucked teat with mouth, then milked by hand	00	00	00
Kid introduced and attempting to nurse but not allowed to take teat in mouth, then milked by hand	00	00	00
Sucked teat with mouth (no milk drawn from teat), with kid present but prevented from nursing, then milked by hand	160	17.3	27.7
Kid nursed vigorously and secured	3		

The second flow of milk induced by the treatment outlined is not quite as great as in the data given in connection with figures 5 and 8, and table 5, but the rise in fat content is greater, and, in this respect, is much the same as the effect noted from the use of pituitrin given in table 5.

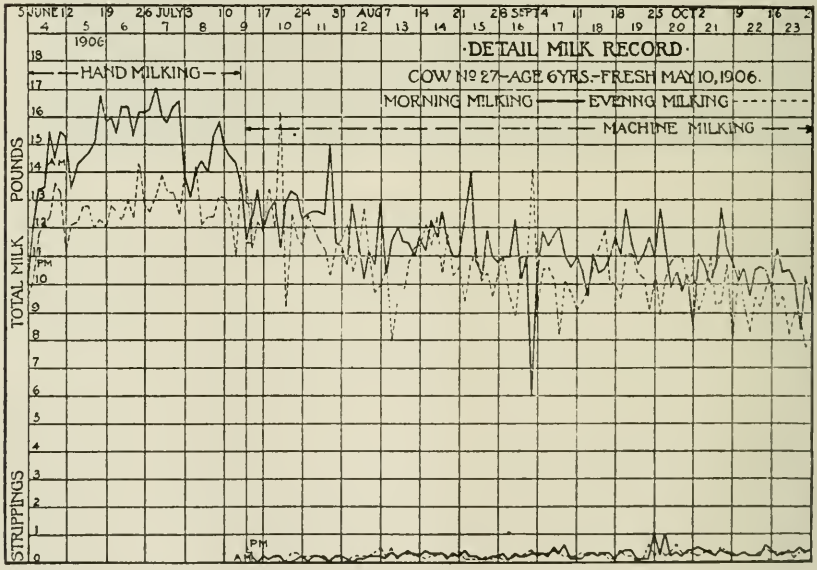

Fig. 9. Showing an illustration of the failure of a milking machine, followed by hand stripping, to secure a uniform degree of removal of the milk from the udder in the cow. Figure from M. S. thesis of the writer, University of Illinois, 1910.

While hand milking does not remove all the milk from the udder that it may be possible to obtain by the use of some stronger stimulus, it usually secures a fairly uniform degree of removal. Thus, in figure

9 showing the yield of milk by a cow after a 13-hour period (morning milking) and after an 11-hour period (evening milking) it is seen that, with hand milking, the curve for the longer period is always above the curve for the shorter period and the two do not cross. However, when machine milking commences, even though it was followed by hand stripping, the character of the curves changes and they cross and intercross repeatedly. This means that the stimulus provided by the machine work did not produce a uniform result. The total yield over a period of some months, it appears, need not be decreased by this condition.

Change in volume of the udder in milking. That the udder of the goat and cow decreases in volume during the process of milking is a matter of common observation. It has been stated [see Marshall (4) p. 557] that the udder of a cow could not contain the quantity of milk which can sometimes be obtained from it at one milking. No quantitative data are given to back up the statement. In making measurements of the change in volume of the udder of the goat by removal of the milk I have found in some cases a decrease equal to the amount of milk secured, and in other cases a decrease less than the volume of milk drawn. An average of four determinations shows a yield of 339 cc. of milk and a decrease in volume of udder of 323 cc. The method of volume determination, as previously noted, was not exact. The results of one trial were as follows:

Goat No. 5. 5/5/15, a.m.

	cc.	cc.
Volume of udder	1540	
Milked out	105	
Kids nursed	255	
Total milk		360
Volume of udder	1170	
Decrease in volume of udder by milking		370
Injected salt solution at 25 cm. water pressure		480
Volume of udder	1635	
Increase in volume		465
Milked out (1.1 per cent fat)	185	
Milked out one hour later (2.5 per cent fat)	82	
Kids nursed	265	
Total fluid recovered (1¼ hours)		532

The pressure of injection used was probably somewhat greater than that at which the milk existed in the udder. It is noteworthy, however, that in this case the decrease in volume of the udder by milk-

ing and nursing was equal to the volume of milk removed. Also, the internal capacity of the udder after milking and nursing was shown to be considerably greater than the volume of milk yielded.[2]

Change in fat content of milk with progress of milking. Data previously given serve to emphasize the well known fact that as milking progresses the fat content of the milk rises greatly. The effect may be duplicated to a certain extent by allowing milk to pass through a quantitative filter paper. The results of such a test are given in table 6. The goat was first turned on her back and the milk cisterns gently massaged to destroy the results of any tendency for the milk to cream in the cisterns. 25 cc. was then drawn, designated as fore-milk; then the main portion,

TABLE 6

Showing the fat content and size of fat globules in fore-milk and stripping milk in comparison with similar data for the main portion of the milking, mid-milk, and two fractions of a 50 cc. portion of the mid-milk separated by allowing to pass through a filter paper until 20 cc. had filtered

SAMPLE	FAT	AVERAGE DIAMETER OF FAT GLOBULES
	per cent.	μ
Fore-milk, 25 cc	4.8	2.2
Strippings, 40 cc	8.3	2.8
Mid-milk, 245 cc	6.0	2.5
Filtrate, 20 cc	5.3	2.3
Non-filtrate, 30 cc	6.6	2.7
Filter control	6.0	
Creaming control	6.0	

245 cc., designated as mid-milk; and finally the last portions, amounting to 40 cc. and designated strippings.

A 50 cc. portion of the mid-milk was placed in a filter and 20 cc. allowed to filter through, requiring about 20 minutes. The portion remaining unfiltered was transferred with the paper to a beaker, the paper gently washed through the milk and removed. This portion is designated non-filtrate. As controls another 50 cc. portion of the mid-milk was treated the same as the above except the unfiltered part

[2] A characteristic reaction of the goats I have worked with is for them to start chewing the cud when milking begins and to continue during the process of milking. Upon the injection of the salt solution in this trial the goat started again to chew her cud and continued as if she were being milked. That is, the passive distension of the gland produced the same reflex, with reference to this particular, as is usually produced during its active contraction during the milking act.

with the paper was added to the filtrate and the paper, after light washing through the milk, removed. This is designated filter control. Still another 50 cc. portion of the mid-milk was allowed to stand in a beaker during the foregoing process and a sample drawn from the surface. This is designated creaming control.

From table 6 it appears that the filter paper retards the passage of the fat globules more than it does the rest of the milk. The fat content and size of the fat globules in the filtrate are decreased. A certain parallelism appears between this effect and that shown in the natural separation of fore-milk and strippings in the removal of milk from the mammary glands. The effect of treating cow's milk (where the fat globules are larger than in goat's milk) as above, is even more marked on the fat content. Thus, in one case a sample of cow's milk containing 7.1 per cent fat showed in the first portion filtered 0.7 per cent fat.

DISCUSSION

It seems highly probable that the remarkable growth of the mammary gland during pregnancy is excited by the presence in the blood of a specific hormone, speaking in terms of current point of view. The evidence of such natural experiments as that of the pygopagous twins, the transplantation experiments of Ribbert, and the foetal extract experiments of Lane-Claypon and Starling, is readily interpreted in such a way. On the other hand it is clear that the hypothetical hormone is not present in large or potent quantities in the blood of the pregnant animal, or else that the mammary gland of the pregnant female is for some reason more susceptible to its influence than the gland of the non-pregnant female; for the transfusion of considerable quantities of blood does not produce positive results. The negative results of Lombroso and Bolaffio with rats united in parabiosis would seem to overthrow the hormone hypothesis. But the authors state that a starved member of such a pair suffered death as quickly as a normal starved control, even though its parabiotic mate was given full feed. That is, there was, apparently, no interchange of circulating food substances. From this it might be well contended that passage of a possible hormone in the blood of the pregnant individual of a pair to the blood of the other, non-pregnant member, was not possible. The fact that lactation may occur in the virgin does not argue against a hormone of pregnancy being *normally* the main factor in the peculiar increased growth activity of the mammary gland during pregnancy. From a teleological point of view

such a hormone, produced by the foetus (or foetal membranes), would be simply an organ for its own post-natal welfare.

While a development of the mammary gland similar to that occuring in pregnancy is not readily produced by the introduction of blood from a pregnant animal into a non-pregnant female, yet such treatment of a lactating animal immediately and temporarily depresses milk secretion. Either inhibition of milk secretion is due to a different hormone than the one causing growth of the gland in pregnancy, or the gland is more sensitive to its influence in the lactating stage. The Lane-Claypon and Starling theory that milk secretion occurs as a result of the removal of this hormone inhibitor, with the removal of the foetus, serves to explain lactation following normal delivery and abortion. The preliminary preparation of the gland, however, seems to be the essential factor for its secretory activity, since lactation ceases normally after a time even though pregnancy does not recur, and, therefore, without the inhibition of any foetal hormone. Further, while lactation is initiated or increased by premature delivery it is not as large in amount as it is following a normal delivery.[3]

The failure of blood transfusion from the actively lactating goat to a feebly lactating one to accelerate the secretion of milk in the latter indicates that lactation is not due to the presence of some particular substance (hormone) in the blood. The fact that one gland may cease lactation before the other also argues against such a factor. The response of the lactating gland to pituitrin is not dependent on the presence of anything in the nature of an activator in the blood; for its non-lactating mate, supplied with the same blood, does not respond. This supports, indirectly, the contention that lactation is not determined by the presence of any exciting substance in the blood stream.

The form of the curves showing the effect of pituitrin on an active, air-inflated mammary gland shows a muscular response. The relatively rapid rise of pressure as expressive of a contraction of the milk passages,

[3] If this point of view is correct it would seem possible that the foetus, by influencing the development and preparation of the gland preceding delivery, might affect the yield of milk by the mother following delivery. Then, the sire of the foetus, by means of the foetus, might influence the milk yield of the mother. In dairy theory and practice, as far as I know, the bull to which a cow is bred is not recognized as a factor in the yield of milk by the cow following the birth of the calf by that mating. Probably the maximum yield of milk is fixed by the genetic endowment of the milk secreting cells of the gland and influences of pregnancy and delivery serve purely a qualitative purpose in initiating activity and have no quantitative effect. The point, however, is worthy of investigation.

followed by the slow decline as expressive of a relaxation is characteristic of the response of smooth muscle. The failure of a second dose to duplicate the effect of the first is not a thing unusual to the action of drugs. Indeed, with pituitrin itself while a first dose causes a rise of blood pressure due to constriction of peripheral vessels, a second dose may not cause a rise of pressure. That the pressure changes in the gland upon injection of pituitrin are not due to changes of blood pressure seems certain from the fact that no change is produced in the non-lactating gland in a late stage of pregnancy, although the gland is at that time highly vascular. That it is not due to a secretory action of the cells is shown by the rapidity of the rise and the return to the initial pressure. A rise of pressure through a secretory action could occur only by the absorbtion of water and its passage into the ducts. If this occurred it is extremely unlikely that resorbtion would commence at once and be carried to a point that would exactly restore the initial pressure. A muscular explanation seems the only one to fit the data. The difference in sensitiveness of the contractile elements of the gland to pituitrin according to the stage of activity finds an analogue in the response of uterine muscle to adrenalin, where the gravid uterus is strongly contracted and the virgin uterus practically unaffected.

The outflow of milk caused by pituitrin is well explained by a muscular action. This seems the more likely as a correct explanation when it is remembered that the act of nursing causes an active increase of pressure in the gland closely resembling the effect of pituitrin. The flow of milk produced by pituitrin when simply placed on the cut surface of the excised gland containing milk shows that its effect is independent of the circulation (except, of course, to bring it in contact with the gland when injected), and gives, also, pretty conclusive evidence that it causes constriction of the very small milk passages of the gland.

Hammond bases his conclusion that pituitrin stimulates the secretory activity of the cells, and has no muscular action on the gland, on the yield of milk obtained by hand milking during a test period of a few hours. Rather positive evidence of a muscular action has been shown above. It must also be clear, from the data given, that any attempt to measure the secretory activity of the gland cells by the amount of milk that may be obtained by hand milking in a test extending over a few hours, is subject to a large error because of variations in the completeness of removal of the milk from the gland. Milk secretion, in the sense of the formation of the milk constituents, is one thing; the ejection of the milk from the gland after it is formed is quite another

thing. The one is probably continuous; the other, certainly discontinuous. And inasmuch as no one has shown any material increase in the milk obtained over a period of days by the use of pituitrin any true secretory action of the drug must be held unproven.

The data given on the flow of milk during milking and nursing show very plainly that there is a reflex constriction of the gland (very similar to that produced by pituitrin) involved; that the removal of the milk from the gland is dependent upon the operation of this reflex; and that the reflex is conditioned. The stimulus which, naturally, excites the reflex must be found in the friction and warmth of the sucking action of the young acting on the cutaneous sense organs of the teat; with, possibly, a further source in the passive dilatation of the sphincter muscles of the nipple by the passage of the milk.

If the mother dog be placed on her back the pups experience no difficulty in getting the full yield of milk, although it must be secured directly against the force of gravity. But if the mother be placed under ether, in a normal position, the pups are unable to secure any milk. Evidently some connection in the reflex arc is broken by the anesthesia, and the indication would seem to be that the central nervous system is involved. The failure of vigorous and continued nursing to secure milk under this condition indicates that the normal removal of the milk is not passive with respect to the activity of the gland. Pituitrin restores the flow, presumably by acting directly on the contractile elements of the gland and producing the same sort of contraction that is ordinarily produced by the nursing reflex.

An artificial imitation of the stimulus afforded by the nursing kid which must have duplicated the natural stimulus very closely, failed to excite the nursing reflex in the absence of the kid; but with the kid present the reflex was called into effective operation by the artificial stimulus as quickly and thoroughly as by the nursing of the kid himself. This may be interpreted to mean that conduction in the reflex arc is dependent upon the psychic condition of the mother.

When the recently delivered cow or mare has been separated from her young some time and her udder is distended with milk, she shows, when brought to her calf or colt, marked symptons of maternal concern, and accompanying this there is often a spurting of milk from the nipples. Apparently, with the gland under this extreme tension, the psychic state produced by the recovery of the young induces a contraction of the gland and an ejection of milk. But in the case of the goat above, with the udder previously drained of milk by hand milking,

recovery of the young, although causing plainly an altered psychic state of the mother, was not sufficient to cause a further flow of milk. But this psychic state in combination with the mechanical and thermal stimuli of nursing permits a reflex more powerful than that excited by the preceding hand milking and calls into play, as it were, a certain reserve strength in the contractile elements of the gland which results in a large flow of milk. In the dog this seems to be the limit of contractile power of the gland, but in the goat pituitrin causes a still further contraction as seen in the ejection of milk. (The statement is based on trials with one goat only.) It is of interest to note the somewhat analogous action of adrenalin on the vascular system. The drug produces a stronger constriction of the blood vessels than can be produced by any reflex nervous excitation; but direct stimulation of the motor nerve to a vessel may cause an equal constriction.

It is difficult to reconcile the seeming dependence of proper nursing or milking on a central nervous mechanism with the results of Eckhard (18) who found no effect on yield of milk in the goat by section of the nerves to the mammary gland; or, with those of Goltz and Ewald (19) who found that removal of the spinal cord in the dog did not interfere with the nursing of her pups.

The determinations of volume change in the udder with milking here reported are hardly extensive or varied enough to be absolute, but so far as they go the indication is that practically the entire quantity of milk obtained at any one time is present as such in the udder at the beginning of milking. Certainly, the capacity of the ducts and alveoli of the udder in the goat is as great as the milk yield at one time. There is, therefore, no *a priori* reason why the milk should not be regarded as being accumulated as such in the udder during the interval between milkings, and the milking operation regarded as a more or less effectual method of stimulating a reflex which causes a contraction of the gland musculature resulting in the ejection of a greater or less part of the milk therein present, according to the effectiveness with which the reflex is excited. It has been claimed that in the heaviest milking cows it is impossible for the udder to contain the quantity of milk that may be secured from it at one time, but that is a matter for determination rather than speculation.

Histologists, as a rule, have been unable to demonstrate any contractile elements in the alveoli of the mammary gland. Positive physiological evidence, however, should outweigh negative histological evidence. Benda (20) has demonstrated contractile elements in the

basement membrane of the alveoli to his satisfaction. The muscular mechanism of the mammary gland seems to be a very important factor in its functioning. It is, indeed, not inconceivable that the activation of the muscular mechanism of the gland may be an important factor in the onset of lactation. Thus, colostrum is secreted by the cells and fills the ducts during pregnancy long before the contractile mechanism is sensitive to pituitrin or to the stimulus of milking. More extensive data than are here available are needed for definiteness, but, at least, it is clear that the contraction of the gland under the influence of pituitrin runs more closely parallel to the appearance of an actual outflow of milk than does the formation of milk (colostrum) by the secreting cells.

The high fat content of strippings milk as compared with fore milk can not be accounted for by a creaming process in the udder for the same phenomenon occurs in the human (Engel (21)). How far the effect noted by simple passing of milk through a filter paper is comparable to the mechanism that causes the result in the mammary gland, is not altogether clear. It is, however, suggestive that the natural process may be partly one of physical retardation of the fat globules in the rapid passage of the milk through the small ducts which must occur in milking. It is generally regarded that the fat of milk is formed within the secreting cells and the globules then extruded through the ruptured end of the cell. It would seem quite possible, also, that under the strain and stress imposed upon the cells by the reflex constriction produced by the milking act this rupture and escape of fat globules from their free ends may be greatly increased and thus cause the higher fat content of the later portions of milk.

SUMMARY AND CONCLUSIONS

Transfusion of blood from a pregnant goat into a lactating one temporarily inhibits milk secretion. Placental and mammary gland extracts from a pregnant cow have an effect similar to the blood. There is some indication of a subsequent accelerating action in the case of the gland extract. Transfusion of blood from a fresh, heavy-milking goat into a low milking one fails to accelerate milk secretion.

Pituitrin has a muscular action on the active mammary gland causing a constriction of the milk ducts and alveoli with a consequent expression of milk. This action holds, also, on the excised gland in the absence of any circulation. The flow of milk produced by pituitrin

is dependent on the amount of milk present in the gland. There is no evidence of any true secretory action. The non-lactating gland, up to a late stage of pregnancy, is not sensitive to pituitrin.

Nursing, milking, and the insertion of a cannula in the teat, excite a reflex contraction of the gland musculature and expression of milk. There is a latent period of 35 to 65 seconds. Milking is a stronger excitant than the cannula; nursing is stronger than milking; [and the direct action of pituitrin (in some cases) is stronger than nursing]. Removal of milk from the gland is dependent upon this reflex, and it may be completely inhibited by anesthesia. The adequate stimulus for the receptor in the reflex arc is the thermal and mechanical effects of nursing; but the strength of the excitation thus aroused is profoundly modified by the psychic state of the mother—especially striking are anesthesia which greatly weakens it, and recovery of the young after separation which greatly strengthens it.

The internal capacity of the mammary gland of the goat is greater than the volume of milk drawn at one time, and the udder shrinks in volume during milking to nearly the same extent as the volume of milk drawn. Practically all the milk drawn is present as such in the gland at the beginning of milking, and is actively ejected by a reflex contraction of the gland musculature under the stimulus of milking.

Passing milk through a filter paper separates it into fractions of different fat content, somewhat similar to fore-milk and strippings. A physical filtration may account, at least in part, for the rise in fat content of the milk with progress in milking.

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AN ANALYSIS OF CERTAIN PHOTIC REACTIONS, WITH REFERENCE TO THE WEBER-FECHNER LAW

I. The Reactions of the Blowfly Larva to Opposed Beams of Light

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I. STATEMENT OF PROBLEM

The problem dealt with in this paper is the analysis of a series of measurements made on the motor reactions of the blowfly larva to light.

The measurements were obtained by a method already used with satisfactory results (8), in which light was applied as two opposed beams the intensity of which could be easily controlled and precisely measured. The reactions produced by two beams of light of different intensities, acting simultaneously on opposite sides of the same animal, were measured in angular deflections from an original path of locomotion. Since no deflection was produced when the opposed lights were of equal intensity, and since a deflection appeared and increased progressively as the intensity difference between the lights was increased, the relative difference between the intensities of the opposed lights was regarded as the value of the stimulus. Some five thousand trails were measured and the results expressed graphically in the form of curves in which the amplitude of the reaction was plotted against the intensity of the stimulus.

The analysis of these curves was undertaken to ascertain, if possible, the factors that determine the relation between reaction amplitude and stimulus intensity. Because there has been considerable work done on the so-called Weber-Fechner relationship between "*sensation value*" and stimulus intensity, it seemed desirable to consider the measurements presented here with reference to the Weber-Fechner Law. Such a method of treatment offers the possibility of interesting comparisons between *reaction* and *sensation* curves, and at the same time gives a definite point of view from which to approach an analysis of the data.

II. INTRODUCTION

The relationship between the strength of a stimulus and the response evoked by it was first systematically studied by Weber about the middle of the last century (11, 12). The criterion by which he measured the effect of the stimulus was "the least detectable change in sensation," a method necessarily limited to human physiology. His experiments showed that the increase in stimulus necessary to cause the least detectable change in sensation is, within a certain medium range, a definite fractional increment of the acting stimulus. Fechner (2) attempted to give this law a more quantitative and extensive application by assuming that just perceptible differences in sensation represent actually equal amounts of sensation. Accepting this assumption, we may express the relation between stimulus and sensation, as determined by Weber's experiments, as follows: in order to increase the sensation by equal amounts, that is, in arithmetical progression, the stimulus must be increased according to a certain factor, that is, in geometrical progression. The sensation may therefore be regarded as a geometrical function of the stimulus. If this relation between stimuli and sensations is represented by a curve in which the ordinates express the sensations increasing by equal amounts, and the abscissas the stimuli necessary to produce them, a logarithmic curve is obtained.

There is doubtless some ground for the assumption of Fechner that least perceptible increments of sensation are equal; but nevertheless it is an assumption, and one that is made in spite of conflicting evidence. We should not, therefore, allow ourselves to be misled by the mathematical treatment of this problem in so far as it is based on an assumption the exactitude of which has not been proved. Waller (9) has pointed out that the matter is further complicated by the fact

that several steps intervene between the external stimulus and the production of the sensation. That is to say, there is first the action of the external stimulus on the end organ; then a nerve impulse in the sensory nerve fiber; and finally the process, in the brain, which gives rise to the sensation. It is a question, therefore, between which of these processes the logarithmic relationship holds good.

While sensation, for the reasons indicated, is an illusive basis for quantitative study, it is the only basis available in the higher forms. Where reactions are profoundly modified by past experience and voluntary control, it is futile to measure them to determine quantitatively the effect of the stimulus, for, instead of being expressed directly in a series of muscular reflexes, a stimulus is then translated first into sensation, and the reaction that follows the sensation may be largely determined by the past experience of the individual rather than by the strength of the stimulus. In such cases, the only thing we can measure is the sensation, or the nerve impulse that causes it.

Hence it is hardly necessary to emphasize the desirability of obtaining additional data from organisms in which the chain of events between the application of the stimulus and the resulting production of some measurable reaction, is not complicated by the factors of sensation and voluntary control based on past experience. If we can eliminate these factors, we may regard the amplitude of the reaction as an index to the intensity of the physiological processes initiated by stimulation. Such reactions are subject to exact physical measurement, and their amplitude may be plotted against the intensity of the stimulus in the form of stimulus-*reaction* curves.

It is only by a careful analysis of many such curves, based on the study of organisms having different chains of physiological processes intervening between stimulus and reaction, that we shall be able to ascertain at what point in the chain of events the approximately logarithmic character of the curves is impressed upon them. For the present it is convenient to speak of reactions presenting a logarithmic curve of increase as following the Weber-Fechner Law, or better the Weber-Fechner Curve. We should, however, be careful to avoid thinking of all the widely divergent types of reactions commonly included in this category as governed by the same law.

A review of the extensive work done on the measurement of sensations does not come within the scope of this paper. The amount of experimental work on the measurement of the amplitude of responses directly controlled by the strength of the stimulus, has been compara-

tively small. There have been many suggestions, incidental to other work, to the effect that such and such an organism, in its response to light or chemical stimulation, apparently conformed with Weber's Law; but in the majority of cases these suggestions have not been based on sufficient data to be of any great value. When therefore measurements of the light reactions of the blowfly larva, made for other purposes, (8), appeared to follow, at least approximately, the Weber-Fechner Curve, it seemed worth while to determine more carefully the exact extent of their apparent conformity with the law, and if possible the causes for it.

The problem in the case of the blowfly larva seemed particularly promising: (1) because the system of measurement, worked out in connection with previous experiments on this form, has yielded consistent quantitative data; (2) because the amplitude of the reaction was directly controlled by the intensity of the stimulus; and (3) because the stimulus reaction curve of a simple reflex of this type would appear to be of considerable value for comparison with the more familar stimulus-sensation curves of human psychology.

III. THE METHOD OF RECORDING AND MEASURING THE REACTIONS OF THE BLOWFLY LARVA TO OPPOSED LIGHTS

In these experiments, the method used for stimulating the larvae and measuring their reactions was the same as that described in det a in an earlier paper (8,pp. 223–239). It is sufficient, here. therefore, tilo outline the procedure very briefly.

The apparatus used was so constructed that the opposite sides of the animal under observation could be subjected to opposed beams of light, the actual and relative intensity of which could be varied at will. Figure 1 shows a plan of the apparatus. It was possible to control the actual intensity of the lights by varying the number of Nernst glowers thrown into circuit (fig. 1, *G*) and by varying their distance (fig. 1, *G f O*) from the observation stage. The relative intensity of the lights was controlled by moving the observation stage from a point midway between the mirrors (where the opposed beams would be of equal intensity) toward one mirror or the other. By calculating the intensities according to the law $I \propto \frac{1}{d^2}$, the distance through which it was necessary to move the stage away from the center, in order to secure any particular intensity ratio between the lights could be readily

ascertained. With the apparatus set up for the desired intensities, larvae were subjected to the stimulation of the opposed beams by forcing them to crawl into the path of light at right angles to a line connecting the mirrors.

In preliminary experiments it became apparent that there was a wide range of individual variability in the photosensitiveness of the larvae. It was not possible to eliminate this factor from comparative measurements by using a single identified larva for each series of tests, because of the shortness of their sensitive period, coupled with the rapid changes in their degrees of sensitiveness with age. The nearest approach that could be made to eliminating the effects of individual variability was to use only those larvae which, by preliminary experiment, were known to react uniformly to a standard test for sensitiveness.

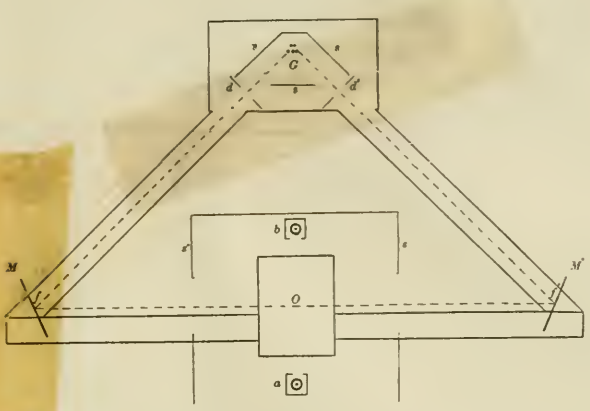

Fig. 1. Diagram of apparatus used to produce differential bilateral light stimulation. *G*, five 220-volt Nernst glowers; *M* and M′, mirrors; *O*, center of observation stage; broken lines, central rays of beams of light from the glowers reflected to *O* by the mirrors; *d* and d′, screens with rectangular openings; *s* and *s′*, light shields; *a* and *b*, 2 c.p. orienting lights with screens.

The standardizing test used consisted in subjecting larvae, which were oriented and crawling under the influence of a horizontal beam of light, to an instantaneous change of 90° in the direction of the beam. Each larva was made to crawl first toward, and then away from the observer, and thus subjected to the change of direction of the light from both its right and left sides. Those larvae orienting immediately and accurately to both changes were considered to be of "standard senstitiveness." The nature of the pairs of trails made by an animal in these tests, and the varying abruptness with which they came into orientation, is shown in figure 2. The data presented in the follow-

ing pages were all obtained from maggots thus standardized for their photosensitiveness, and they may therefore be considered reliable for comparative study.

These standardized larvae were subjected to stimulation in the following manner. Each maggot was allowed to crawl onto the observation stage immediately in front of the orienting light (fig. 1, a). Under the influence of this light it was forced to crawl toward the center of the stage in a direction at right angles to the path of light thrown by the mirrors (fig. 1 ff′). By putting a drop of dilute methylen blue on its posterior end, the larva was made to record its own trail on a sheet of paper. When the animal was well onto the stage and crawling steadily, the orienting light was turned out and the mirror

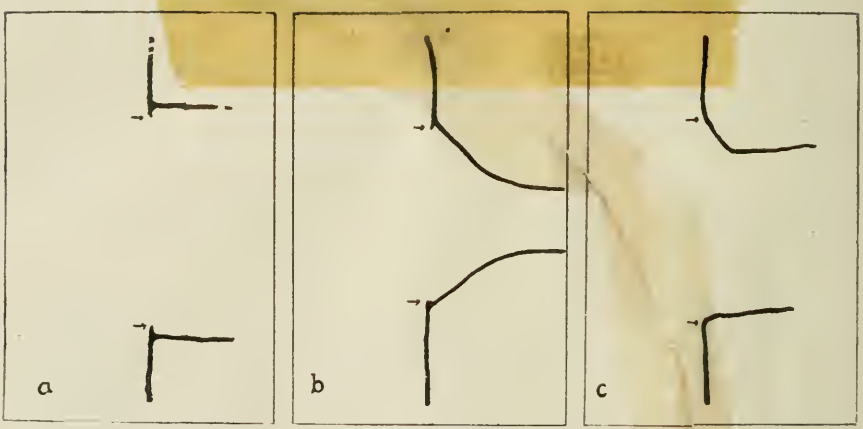

Fig. 2. Three examples of test trials which pass the standard orientation test. Each pair of trails was made by a different larva. Though there is some difference in the sharpness of orientation, each trail comes to lie parallel to the new direction of light. The arrows mark the points at which the direction of the light was changed.

beams were thrown on simultaneously from either side, the point at which the larva was subjected to the bilateral illumination being indicated on the trail. The part of the trail crawled under the influence of the bilaterally applied stimulus is the significant portion.

Loeb (5, p. 2), has pointed out that when "two sources of light of equal intensity and distance act simultaneously upon a [*negatively*] heliotropic animal, the animal puts its median plane at right angles to the line connecting the two sources of light." We should expect, then,

that a larva subjected to the action of opposed beams of equal intensity, would continue crawling in a direction at right angles to a line connecting the mirrors. That such is in fact the case was demonstarted by a large number of trials. It was, however, observed that certain larvae tended to crawl somewhat toward either their right or left even under balanced illumination. Apparently this tendency to deflect from the normal course was due to bilateral asymmetry of photosensitiveness (8). To eliminate the distorting effect of unbalanced sensitivity from the final measurements of the trails, each larva was started onto the stage first from one direction and then from the other. By thus reversing the direction of crawling and making the trails in "opposite-direction-pairs," the deflection due to

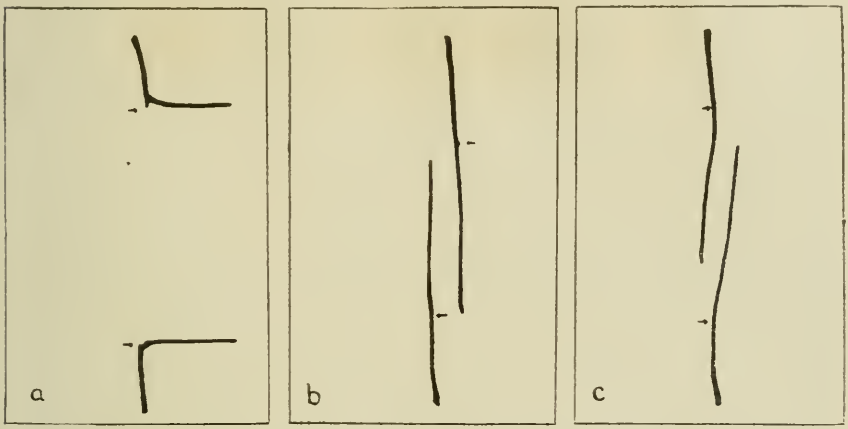

Fig. 3. A set of trails made under equal, opposed lights by a symmetrically responding larva; *a*, the test trails; *b* and *c*, trails under the influence of balanced lights.

asymmetry was thrown on opposite sides of the "norm," and would consequently be canceled out of the final measurements. Figure 3 is a photograph of the complete record of a larva's responses to equal bilateral stimulation; *a* shows the pair of trails made by the maggot in the preliminary standardizing test; *b*, an "opposite-direction-pair" of trails made under balanced illumination; and *c*, a second pair of trails similar to those in *b*, made after the larva had been rested for half an hour. In the record sheet photographed in figure 3, *c*, it will be observed that the trails do not conform exactly to the perpendicular to the line connecting the sources of light. There is a deflection to

the right in that trail made by the larva while crawling away from the observer, and to the left in that trail made by the same larva while crawling toward the observer. If now we measure the deflection of the trails in degrees, by placing a protractor with its center at that point on the trail where the head of the larva was when the lateral lights were turned on, and its base parallel to the line connecting the sources of light (fig. 1, ff') we find that the deflections to the right and left are of equal value. By tabulating left hand deflections in *plus* degrees and right hand deflections in *minus* degrees, the effects of asymmetry are cancelled, and we have as a final result a measurement identical in value with those measurements taken on larvae that have a perfect balance of sensitiveness. As a certain amount of unbanance of sensitiveness is far more common than perfect symmetry, this checking out in all measurements of the possible effects of asymmetry is of the utmost importance. By taking the sum of the plus and minus deflections of a larva, and dividing it by the number of trails measured, the *average angular deflection* of the larva is determined. The average obtained by measuring four trails such as those shown in figure 3, *b* and *c*, expresses very accurately the response of any individual larva.

The mean of the *average angular deflections* of a number of larvae may be considered as expressing accurately the value of the response which is elicited by a given set of conditions. Table 1 is an example, taken from several similar sets of measurements that were made under equal bilateral illumination, which serves to show the method of compiling the measurements. A study of the table shows that very few trails conform exactly to the theoretical response, but the total plus and minus deflection of each larva comes near to cancelling, and the average of the four trails of each larva is close to zero degrees deflection. The average response of the whole set corresponds almost exactly to the theoretical response, the average deflection from the perpendicular being only $-0.025°$. This is astonishingly accurate when one considers that a degree on the protector of 7.6 cm. radius used in measuring the trails was only 1.5 mm.

The striking results of table 1 were confirmed by several repetitions of the experiment. The immediate significance of these results is two-fold. (1) The consistent closeness of the average trail to the perpendicular, and the equal distribution of the trails on either side of it, indicate that the method of individual measurement has eliminated from the final results the effects due to asymmetry and placed the different individuals on a uniform basis for comparison. (2) The close

conformity of the aggregate response to the theoretical response, when the lights are of equal intensity, makes a well grounded point of departure for a series of experiments with opposed lights of unequal intensities; for if there is no deflection toward either side following equal bilateral stimulation, the deflection appearing under unequal bilateral simulation may be regarded as a true expression of the physiological effect of the difference between the lights. Having, therefore, perfected a system of measuring the reactions of the larvae to light and established a fixed point of departure by the measurements under equal opposed lights, we were in a position to measure the reactions to a graded series of intensity ratios.

TABLE 1

A table giving the values of the angular deflections made by larvae under the stimulation of equal opposed lights

NUMBERS OF THE LARVAE / DATE	1ST SHEET		2ND SHEET		− TOTAL	+ TOTAL	NET	AVERAGE ANGULAR DEFLECTION
	1st trail	2nd trail	3rd trail	4th trail				
								degrees
No. 14, 13/5/'13	+10	−16	+15	− 7	23	25	+ 2	+0.50
No. 18, 13/5/'13	0	+ 6	+ 2	+ 5	0	13	+13	+3.25
No. 20, 13/5/'13	+ 7	− 8	+18	−12	20	25	+ 5	+1.25
No. 15, 13/5/'13	+25	−11	−11	− 3	22	28	+ 6	+1.50
No. 4, 13/5/'13	+ 6	− 6	+ 9	− 7	13	15	+ 2	+0.50
No. 22, 13/5/'13	0	+ 5	− 7	− 1	8	5	− 3	−0.75
No. 21, 13/5/'13	− 5	− 3	−21	+17	29	17	−12	−3.00
No. 16, 13/5/'13	+17	−14	0	− 6	20	17	− 3	−0.75
No. 11, 13/5/'13	− 9	− 1	+13	− 6	16	13	− 3	−0.75
No. 9, 13/5/'13	−11	+ 5	− 5	+ 3	16	8	− 8	−2.00
10 larvae 40 trails					167°	166°	−1°	−0.025

IV. A COMPARISON OF THE STIMULUS-REACTION GRAPHS OF THE BLOWFLY LARVA WITH THE WEBER-FECHNER CURVE

1. The graph expressing the values of the angular deflections produced by a graded series of percentage differences of intensity between opposed lights.

The problem, so far as the method of experimenting was concerned, now resolved itself into the compiling of a series of reaction measurements for comparison with various values of the acting stimulus. As

has already been pointed out, we proposed to measure, not sensations, but the amplitudes of reflexes initiated by stimuli of various degrees of intensity. The measurable reaction in the method of experimenting outlined above, is the larva's *angular deflection* from an original path of locomotion. The original path of locomotion was so arranged with reference to the lights producing the significant portion of the reaction, that the larva came into the path of the light at right angles to the line connecting their sources. We have already seen (Table 1, p. 321) that when the opposed beams were of equal intensity, there was no deflection from the initial direction of locomotion. But if we subject larvae to the action of *unequal* lights, there appears a deflection toward the weaker light, which is directly proportional to the difference of intensity between the lights (Table 3 and figure 4). We may say, therefore, that

TABLE 2

Summary of measurements of 200 trails made to determine the value of the angular deflection when the opposed lights are of equal intensity

EXPERIMENT NUMBER	NUMBER OF TRAILS MEASURED	TOTAL MINUS DEFLECTION	TOTAL PLUS DEFLECTION	NET DEFLECTION	AVERAGE ANGULAR DEFLECTION
		degrees	*degrees*	*degrees*	*degrees*
1	40	187	165	−22	−0.55
2	40	180	176	− 4	−0.10
3	40	207	225	+18	+0.45
4	40	167	166	− 1	−0.025
5	40	246	237	− 9	−0.225
Total.	200	Average angular deflection for 200 trails −0.09			

the angular deflection of the larva is made in response to a differential bilateral stimulus, the value of which may be expressed in terms of the relative intensity of the opposed lights, or more conveniently, in terms of the percentage difference between the lights.[1]

The measurements obtained from the trails of larvae subjected to equal bilateral stimulation were taken as the starting point of the stimulus-reaction curve. Table 1 shows in detail the deflection measurements taken from a set of 40 trails made by ten larvae. This set of data was supplemented by four other similar sets. Without reproducing the tables in detail, the results may be summarized as in Table 2. The totals of Table 1 appear in Table 2 as experiment 4.

[1] The percentage differences throughout the experiments are computed in terms of the *stronger* light.

Stating the results of Table 2 in terms of the series of experiments of which it forms the starting point, we may say that the reaction to opposed lights of 0 per cent intensity difference, is a minus deflection 0.09°. This is so near zero degrees that it undoubtedly signifies the absence of deflection under these conditions.

Following the experiments at equality, a series of measurements was made at the following percentage differences between the lights: $8\frac{1}{3}$ per cent, $16\frac{2}{3}$ per cent, 25 per cent, $33\frac{1}{3}$ per cent, 50 per cent, $66\frac{2}{3}$ per cent, $83\frac{1}{3}$ per cent, and 100 per cent. As the method of measurement and tabulation was the same throughout, a summarizing table will be sufficient to show the results obtained.

TABLE 3

A table based on the measurement of 2,500 trails showing the progressive increase in angular deflection with increasing differences between the lights.

Percentage Difference in Lights.	Equality 0%	$8\frac{1}{3}$%	$16\frac{2}{3}$%	25%	$33\frac{1}{3}$%	50%	$66\frac{2}{3}$%	$83\frac{1}{3}$%	100%
Number of Trails Measured.	200	200	200	500	500	500	100	100	200
Average Angular Deflection.	−0.09	−2.77°	−5.75°	−8.86°	−11.92°	−20.28°	−30.90°	−46.81°	−77.56°

These results may be expressed graphically by plotting degrees of deflection along the axis of ordinates and percentage differences of intensity along the axis of abscissas. The stimulus-reaction curve thus obtained is shown in figure 4.

It is obvious that this curve is not directly comparable with the logarithmic Weber-Fechner Curve constructed on the basis of *absolute* increments in the stimulus, since the curve of figure 4 is plotted on the basis of *percentage* increments in the stimulus. For purposes of comparison, however, a theoretical curve may be constructed of the type which would be obtained by measuring, under the conditions of these experiments, a reaction governed by the Weber-Fechner Law. Using percentage differences in stimulus instead of absolute differences, and degrees of deflection instead of sensation units, such a curve has been plotted as a broken line in figure 4.

The construction of this theoretical curve is based on the following simple deduction from the usual statement of the Weber-Fechner law. If "the increase of stimulus necessary to produce a unit increase in response is a definite fractional increment of the acting stimulus," then it follows that the same fractional increment *in a stimulus of any*

intensity will produce equal changes in response. In these experiments (see p. 322) an increase of the intensity difference between the opposed lights is regarded as an increase in the stimulus acting to deflect the larva from its original path of locomotion. For a difference of $8\frac{1}{3}$ per cent between the lights, there is experimentally a deflection of 2.77° (see fig. 4 and Table 3). Dealing in round numbers let us assume, for the plotting of the theoretical response, that a difference of $8\frac{1}{3}$ per cent

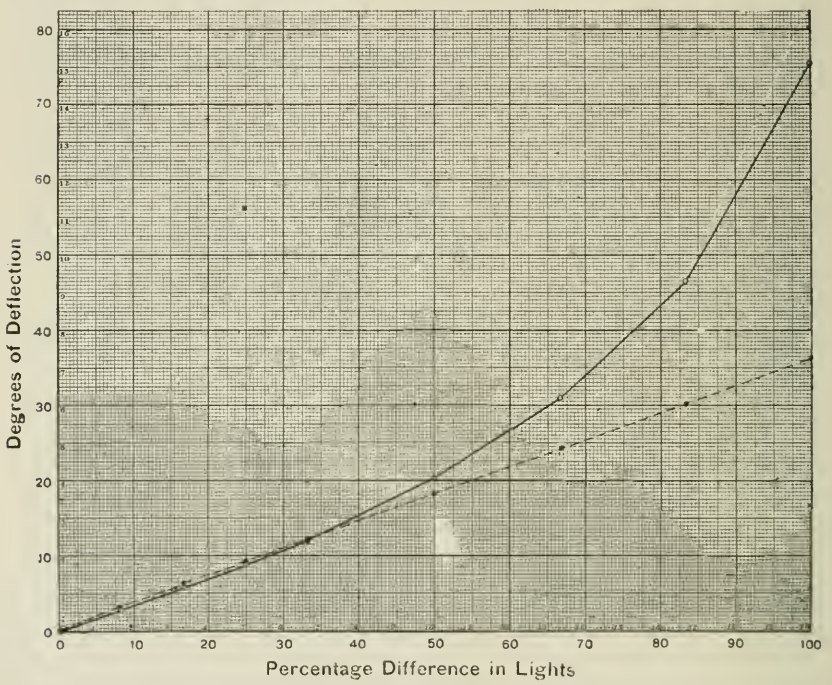

Fig. 4. The solid line is a curve representing the angular deflections of the blowfly larva under opposed lights of various intensity ratios. (See also table 3). The broken line is a theoretical curve such as would be obtained by plotting, under the same conditions of measurement, a reaction governed by the Weber-Fechner Law. In plotting this theoretical curve, a difference of $8\frac{1}{3}$ per cent between the lights is assumed to produce a deflection of 3°.

Ratio of Lights.

1 to $\frac{11}{12}$ = $8\frac{1}{3}$% difference.
1 to $\frac{5}{6}$ = $16\frac{2}{3}$% difference.
1 to $\frac{3}{4}$ = 25% difference.
1 to $\frac{2}{3}$ = $33\frac{1}{3}$% difference
1 to $\frac{7}{12}$ = $41\frac{2}{3}$% difference.
1 to $\frac{1}{2}$ = 50% difference.

between the lights will produce a deflection of 3°. This point would be located in precisely the same manner as the first point on the logarithmic Weber-Fechner curve. It is after the location of the first point on figure 4 that the method of plotting this percentage-increment curve begins to differ from that of plotting the absolute-increment curve. In the former curve, each point must of necessity be figured from unity as a starting point; in the latter, only the first point is so figured; each succeeding point being based on the one immediately before it. The values of the stimuli in an absolute-increment series would run: $1 + \frac{1}{12}$ of 1 equals 1.083; $1.083 + \frac{1}{12}$ of 1.083 equals 1.173; $1.173 + \frac{1}{12}$ of 1.173 equals 1.271; etc. The values in the percentage-increment series would run: 1 to $\frac{11}{12}$ ($8\frac{1}{3}$ per cent difference); 1 to $\frac{5}{6}$ ($16\frac{2}{3}$ per cent difference); 1 to $\frac{3}{4}$ (25 per cent difference); 1 to $\frac{2}{3}$ ($33\frac{1}{3}$ per cent difference); etc.; computed each time from the same starting point (unity). Each increase of $8\frac{1}{3}$ per cent in the difference between the lights is assumed, in this case, to produce an increase in deflection of three degrees. The theoretical curve plotted in this way is rectilinear (fig. 4).

The steps in this percentage-increment curve do not represent the same sort of intervals as the least perceptible increments used by Fechner. In experimental work of the type under consideration it is not practical to use least perceptible changes in reaction as units of measurement, for it involves, to too great an extent, the personal judgment of the experimentor. It is far more accurate to measure responses that are of readily appreciable amplitude by some standard unit of physical measurement. If it is desirable to compare the results thus obtained with results obtained by typical Weber-Fechner measurements, it is possible to put the two types of curves on the same mathematical basis. Suppose the Weber-Fechner unit of reaction measurement to be represented by the letter u. A response which is of considerably greater amplitude than the least detectable change in reaction may be represented as n times u. If there is justification for considering least perceptible increments as units, then we may handle $n.u$ in any formula in which u could be handled. If the Weber-Fechner unit is not reliable for quantitative work, as much experimental evidence seems to indicate, we have, by using standard units of measurement put the whole matter on a more sound quantitative basis. The curve of figure 4, though constructed on a different basis from that therefore employed in work of this nature, may be regarded as expressing the stimulus-response relationship postulated by the Weber-Fechner Law.

Because it is based only on the value of a step at the beginning of

the experimental series, the curve shown in figure 4 is not necessarily correct for the conditions of the entire set of measurements. It is, nevertheless, evident that a curve expressing the Weber-Fechner relationship plotted to percentage increase in stimulus would be rectilinear. The reason for the *apparent conformity* of the larva's stimulus–reaction curve with the theoretical curve through the range of lower intensity differences, is that the theoretical Weber-Fechner Curve was constructed on the basis of units taken from the lower portion of the experimental curve. Clearly there can be no conformity, other than a limited fortuitous one, between the theoretical rectilinear curve of the Weber-Fechner Law and the experimental stimulus-reaction curve of the blowfly larva.

2. The graphs expressing the values of the angular deflextions produced by a graded series of absolute intensities of opposed lights having their relative intensity maintained constant.

Under the experimental conditions already described, it was possible to make another series of measurements, which may be considered from the point of view of the Weber-Fechner Law.

Granting for the present Fechner's assumption that just perceptible differences in sensation may be considered as equal differences,—or sensation units,—we may make the deduction from the law already referred to on page 323, namely: if the increase of stimulus necessary to produce the least perceptible increase of response is a definite fractional increment of the acting stimulus, then it follows that, within physiological limits, a definite fractional increment of a stimulus of any intensity would produce the same change in reaction. To put it concretely, a $33\frac{1}{3}$ per cent addition to a stimulus of one unit should produce the same increment in reaction as a $33\frac{1}{3}$ per cent addition to a stimulus of five units, though the absolute increase in stimulus is $\frac{1}{3}$ of a unit and $\frac{5}{3}$ of a unit in the other.

To obtain data which could be referred to this form of the Weber-Fechner law, it was necessary to maintain the *intensity ratio* of the opposed lights constant, while the lights were varied, together, through a series of different absolute intensities. Such conditions were readily producible with the apparatus described on page 317. The intensity ratio of the two lights at any point between the mirrors (fig. 1, *M* and M′) is determined by the distances, along the path of the beams, from that point to the source of light. Therefore if the observation stage is fixed at the point where a desired intensity ratio exists, the absolute intensity of the common source of light (and with it the intensity of

the opposed beams) may be varied at will without changing the intensity ratio of the lights at the observation stage.

In these experiments the apparatus was set up for intensity ratios, corresponding to zero, $8\frac{1}{3}$, $16\frac{2}{3}$, 25, $33\frac{1}{3}$ and 50 per cent differences between the opposed lights. At each of these intensity ratios the common source of light was varied by using from one to five Nernst glowers. Table 4 gives the actual intensities of the opposed lights under the above conditions, measured at the center of the observation stage.

TABLE 4

The intensities of the opposed beams of light measured in candle meters at the center of the observation stage

PERCENTAGE DIFFERENCE IN LIGHTS	RATIO OF LIGHTS	INTENSITY OF WEAKER LIGHT IN CA. METERS					INTENSITY OF STRONGER LIGHT IN CA. METERS				
		1 glower	2 glowers	3 glowers	4 glowers	5 glowers	1 glower	2 glowers	3 glowers	4 glowers	5 glowers
Equality	1 to 1	6.32	13.9	24.6	31.5	41.1	6.32	13.9	24.6	31.5	41.1
$8\frac{1}{3}$%	11 to 12	6.05	13.4	23.6	30.2	39.3	6.61	14.6	25.7	32.9	42.9
$16\frac{2}{3}$%	5 to 6	5.77	12.8	22.5	28.8	37.5	6.93	15.3	26.9	34.5	45.0
25%	3 to 4	5.50	12.1	21.4	27.4	35.7	7.34	16.2	28.5	36.5	47.6
$33\frac{1}{3}$%	2 to 3	5.21	11.5	20.2	25.9	33.8	7.83	17.1	30.5	39.0	50.9
50%	1 to 2	4.60	10.2	17.9	22.9	29.9	9.20	20.2	35.8	45.9	59.9

The reactions of the larvae to the five different absolute intensities used at each intensity ratio of the opposed beams, were measured and the data compiled in Table 5. The same results have also been plotted

TABLE 5

A table based on the measurements of 2700 trails, showing the average angular deflections at five different absolute intensities and six relative differences of intensity

	EQUALITY	$8\frac{1}{3}$ PER CENT	$16\frac{2}{3}$ PER CENT	25 PER CENT	$33\frac{1}{3}$ PER CENT	50 PER CENT
	degree	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*
1 Glower	−0.55	−2.32	−5.27	−9.04	−11.86	−19.46
2 Glowers	−0.10	−3.05	−6.12	−8.55	−11.92	−22.28
3 Glowers	+0.45	−2.60	−5.65	−8.73	−13.15	−20.52
4 Glowers	−0.025	−2.98	−6.60	−9.66	−11.76	−19.88
5 Glowers	−0.225	−2.925	−5.125	−8.30	−10.92	−19.25
Average	−0.09	−2.77	−5.75	−8.86	−11.92	−20.28

graphically in the form of a five-point curve to express the values of the reactions obtained under each of the six intensity ratios. (fig. 5).

If the principle of the Weber-Fechner Law holds good, a definite percentage change in the value of any acting stimulus will produce the same change in reaction (see p. 326). In these experiments, therefore, the Weber-Fechner expectation is a horizontal rectinilear curve at each intensity ratio. All the experimental curves, however, show marked unevenness. Take for example the curve obtained under a $33\frac{1}{3}$ per cent difference of intensity. There is a maximum deflection of 13.15° at the three-glower intensity, and a minimum deflection of 10.92° at the five-glower intensity. The curve, however, is of a roughly hori-

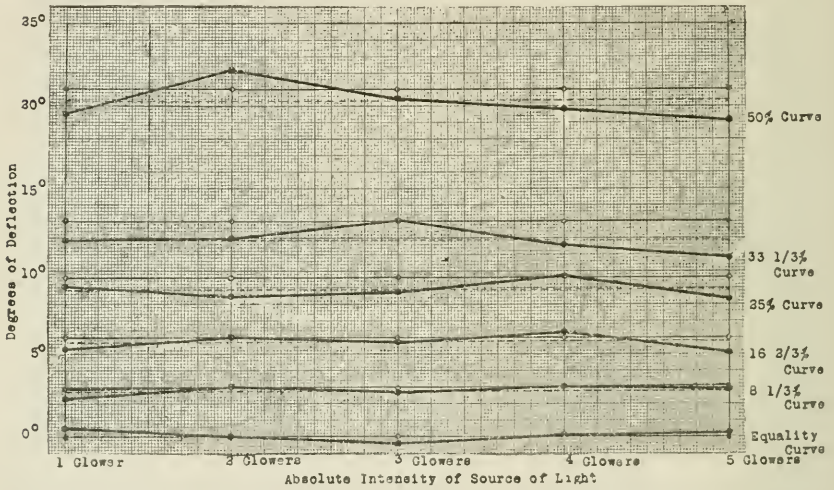

Fig. 5. Curves showing angular deflection made by blowfly larvae under opposed lights maintained at a fixed intensity ratio, while the absolute intensity was varied by using from 1 to 5 Nernst glowers at the source of light. The broken lines represent the average position of the 5 points of each curve. For significance of solid, horizontal lines see page 337.

zontal, rectilinear type. The interpretation of the results depends on how much significance we attach to the variation of 2.33° between the points of the curve most widely different in value from each other. Cases in which a moderate intensity of light, gave a greater response than a very high or very low intensity, have been observed by Walter for Planaria (10) by G. P. Adams for Allelobophora (1), etc., which made me rather expect that a similar maximum at a medium intensity might

appear in the curves run at other percentage differences. This, however, was not the case, as may be seen from the graphs of the reaction values at six different ratios of intensity (fig. 5). There is no consistency in the position of the maxima; in fact, no two curves are strikingly alike in any respect, save possibly the tendency to run low at the five-glower intensity. As the maxima depart but little from the mean, such inconsistency among the similar curves suggests that the variations are probably merely fluctuations due to experimental error. This is further indicated by computing the average position of the five intensity points of a curve and comparing the amount by which the maximum or minimum is separated from the mean, with the aver-

TABLE 6

A table showing the interrelation of percentage difference in the intensity of the the lights, average angular deflection, average error of the trails, and departure of the maximum from the mean of the curve.

RELATIVE INTENSITY OF THE LIGHTS	AVERAGE ANGULAR DEFLECTION	"AVERAGE ERROR"—AVERAGE DEVIATION OF SINGLE TRAILS FROM MEAN OF THE SET	GREATEST DEPARTURE OF ANY POINT ON CURVE FROM MEAN OF CURVE	GREATEST DEPARTURE FROM THE MEAN OF THE CURVE EXPRESSED IN % OF THE AVERAGE DEVIATION
	degrees	*degrees*	*degrees*	*per cent*
Equality	0.09	±1.98	0.53 (at 3 gl.)	27
8⅓% difference	2.77	±1.58	0.45 (at 1 gl.)	28
16⅔% difference	5.75	±1.87	0.85 (at 4 gl.)	41
25% difference	8.86	±2.93	0.80 (at 4 gl.)	27
33⅓% difference	11.92	±3.36	1.23 (at 3 gl.)	36
50% difference	20.28	±4.75	2.00 (at 2 gl.)	42

age variability of the trails used in compiling the curve. This comparison shows the departures of the maximum and minimum to lie well within the value of the "average error" of the experimental data.

Take, for example, the case of the curve which has the greatest variation, the curve at 50 per cent difference. The deflections (see Table 5 and fig. 5) are at one glower, 19.46°; two glowers, 22.28°; three glowers, 20.52°; four glowers, 19.88°; five glowers, 19.25°. The average deflection for all five intensities is 20.28°, represented on the 50 per cent curve of figure 5 by the (broken) "mean line." The greatest departure from this mean is at the two-glower intensity. Now by referring to Table 6 the average deviation of single trails from the mean trail at 50 per cent difference is seen to have been ±4.75°. The maximum

departure of this curve from the mean is but 42 per cent of the average error of the experimental data on which the curve was based. Similar computations have been made for the other curves and the figures given in Table 6. The departures of the curves from a straight line are not only well under the value of the experimental variation in all cases, but they bear a reasonably constant relationship both to the average error and to the total deflection at each intensity. As the amount of response diminishes in the successively smaller intensity differences, the average departure of the trails from the mean diminishes. And as the average error of the trails decreases, the separation of the maximum from the mean decreases correspondingly. The last bit of evidence is the variation in the curve at equality. This falls in place in the decreasing series, but yet the variations from the mean are still sufficient to indicate the fortuitous nature of the similar variations in the other curves, for if an absolutely straight line could be obtained under any conditions, it would be at equality. There seems, therefore, to be no room to doubt that the slight maxima of these curves are merely chance variations, and that the significance of each curve is a horizontal line represented by the "mean line" computed from the five points of a curve. The manner in which the curves of the series grade in together without overlapping, and the confirmation of each curve by every other curve, make the evidence practically complete.

We may therefore conclude that the curves of angular deflection values for a graded series of absolute intensities of opposed lights having their relative intensity maintained constant, resemble in contour the rectilinear curves expressing the Weber-Fechner expectation. The significance of this resemblance is a matter which is taken up at length in the subsequent discussion of the results. It is puzzling in view of the fact that the other series of measurements made on the same animal under the same experimental conditions can not in any way be regarded as following the contour of the theoretically correct Weber-Fechner Curve.

V. DISCUSSION

Apparently it is customary to consider a given reaction to be in accord with the Weber-Fechner Law, when the curve representing the magnitude of the responses produced by increasing stimulation is similar to a certain logarithmic curve said by Fechner to express the stimulus sensation relationship which Weber established for the sense of touch

in man. Astonishingly diverse types of reactions have been held to yield such a curve; the category runs all the way from the photic reactions of fungi (6) to human sensations. The heterogeneity of the reactions said to follow this law has occasioned strangely little comment. It seems hardly more justifiable to assume, without inquiring into the mechanism of the responses, that widely different organic reactions follow the same law because they exhibit similar curves relating reaction amplitude to stimulus intensity, than to classify animals on the basis of their size.

Critical consideration of the various responses said to follow Weber's Law will, I believe, lead to one of two conclusions: either there is some phenomenon fundamental to all these types of reaction which impresses its characteristics on the responses in which it occurs, whether or not they involve a central nervous system, or we have been misled by the lure of a mathematical formula and have sheltered under it a group of observations only superficially similar. Which of the conclusions is correct can be judged only in the light of further and more closely analytical work. It is not improbable that both conclusions may prove to a certain extent true. When the various reactions said to follow Weber's Law have been successfully analyzed, and when it is known *why* each reaction follows the curve that it does, it may be possible to say that a certain group of reactions follow a logarithmic course *because* there is some particular phenomenon common to them all. Quite probably the same analyses which make possible the formulation of a law governing such a group of reactions will exclude from the group certain reactions presenting a curve which is similar, but where the similarity results from causes peculiar to the individual responses. At present there is little justification for talking about a "psychophysical *law*." It can be said, only, that many reactions have been found to exhibit strikingly similar curves when increase in their amplitude is plotted against increase in stimulation. When the causes for this similarity are known, we will be in a position to formulate a law—or to abandon an old hypothesis.

The experiments dealt with in this paper were arranged to facilitate a consideration of the results from the point of view of the Weber-Fechner Law. It was hoped that if these stimulus-reaction curves did conform with the curves obtained in stimulus-sensation work, analysis of the different reactions might show a dominant factor common to both types of response. The establishing of such a factor would go a long way toward clearing up our rather hazy ideas as to the nature

of the so-called "psychophysical relationship." As we have seen, however, of the two series of measurements made on the same animal by the same experimental methods, one clearly does not accord with the Weber-Fechner Curve, whereas the other way may be regarded as following, within the limits of experimental error, the theoretic expectation of the law. If Weber's law held good for this reaction it would govern the reaction from whatever point of view the measurements were considered. Since such is not the case, we must seek some factor which is capable of producing both of the two types of curves obtained experimentally. Such a controlling factor is, I believe, demonstrable in the structural plan of the orienting mechanism of the larva.

In an earlier paper, experimental evidence was advanced which justifies the conclusion that, in this form, orientation to opposed lights depends upon symmetrically located sensitive areas operating bilaterally on the musculature (8). The evidence may be summarized as follows: (1) When the lights acting on the opposite sides of the larva are equal, the larva orients so that its median plane is at right angles to the line connecting the sources of light. (2) When the opposing lights are unequal, a deflection toward the weaker light appears. (3) Certain larvae are asymmetrical in their response, deflecting toward the less sensitive side when subjected to equal bilateral illumination. (4) The blackening of one side of the sensitive region produces a deflection toward the side thus artificially made less sensitive (3). (5) Asymmetry of sensitiveness may be balanced by a corresponding inequality of the stimuli acting on opposite sides of the animal.

In view of these facts there seems to be no explanation for the responses of the blowfly larva to opposed lights, other than the assumption that symmetrical sensitive areas affect the musculature of the two sides of the animal in proportion to the stimulation received. We do not know precisely the nature of the mechanism concerned in the reaction, but there are certain general lines on which such a mechanism must be based. "If the angle of orientation under opposed beams of light is such that the stimulation of the opposite sides is equalized, the receptive mechanism must be of such a nature that varying the axial position of the animal produces changes in the relative amount of stimulation received on opposite sensitive areas. Otherwise there would be no cause for the animal to assume a definite angle of orientation for each intensity difference between opposed beams of light" (8).

This equalization cannot be accomplished by bilaterally located sensitive areas that are parallel to each other. Change in the axial posi-

tion of an animal having localized, bilaterally placed, parallel photo-receptors, would result in a change of the intensity operating upon the sensitive surfaces of each side of the animal, but since the surfaces are parallel their projection on a plane at right angles to the rays of light would be the same whatever the axial position, and consequently the amount of light falling on the two sensitive surfaces would be always equal. If, however, we assume that the sensitive surfaces are inclined at an angle to each other, the case is different. Changes in the animal's axial position with reference to the lights will then result in changes not only of the effective illumination on both sensitive surfaces, but in the relative intensity of the light operative on the opposite photo-sensitive areas. It is not unjustifiable to assume as a working hypothesis, therefore, that the power to balance unequal bilateral stimulation, by a change in the position of the axis of the body, depends on the inclination to each other of the photo-sensitive surfaces.

It is possible to compute the value of an angle of sensitive surfaces which would equalize the light stimulation on the opposite sides of an animal, when the angle of orientation the animal assumes under unequal bilateral illumination is known. This has been done in the case of the blowfly larva, on the basis of angles of orientation experimentally obtained under four different intensity ratios of opposed lights.[2] The magnitudes of the four angles thus constructed are in good agreement, being 82°, 83°, 83°, and 82°. The consistency of these computations, together with the fact that some mechanism operating after the manner of sensitive areas inclined to each other at an angle seems to be the only way in which unequal bilateral stimulation can be equalized, led me to put forward the hypothesis outlined. I believe it expressed in a crude and much simplified form the dynamics of orientation in the blowfly larva.[3]

It seemed to me possible that the structure of the reacting mechanism might in this case, be the factor which determined the amplitudes of the responses to various values of the stimulus. To test this supposi-

[2] For a detailed discussion of the method of constructing the hypothetical angle, and the geometrical proof of the construction, see (8) pp. 265–268.

[3] This angle, I would emphasize again, probably does not represent the actual position of the sensitive surfaces in the larva. There are too many modifying factors intervening between the direction of the rays of light in the field and the angle of incidence of the light on the sensitive areas to permit of basing any conclusions directly on the magnitude of the computed angle, for the structural peculiarities of the dioptric apparatus, or of the tissue overlying the sensitive area would change its value to a large extent.

tion, an angle of sensitive surfaces was assumed corresponding to the value of the angle computed in the paper referred to. Taking 80° as the angle of inclination of the sensitive surfaces to each other, the angle of orientation which would equalize the stimulus bilaterally was calculated for the intensity ratios used in this series of experiments. The angular deflection values thus obtained were plotted graphically for comparison with the experimental curve (figure 6.) The two curves correspond so exactly that they are indistinguishable at several points.

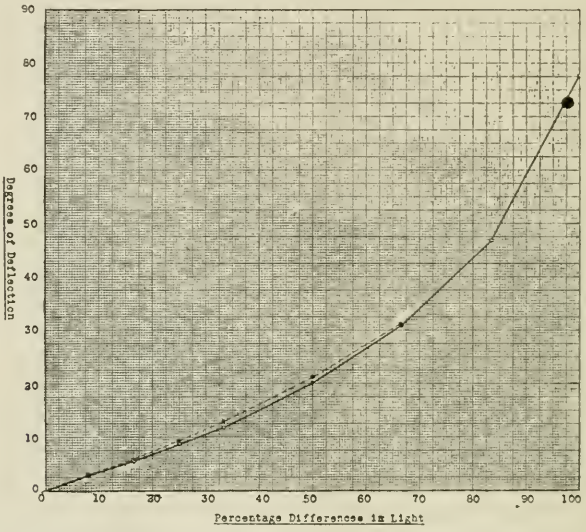

Fig. 6. A figure for the comparison of the reaction graph of the blowfly larva, with a graph showing the angular value of the axial positions that would equalize the light bilaterally in a photoreceptive system with sensitive surfaces inclined at an angle of 80° to each other. Solid line, experimental curve (see fig. 5); broken line, theoretical curve.

It will be observed, however, that the theoretical curve has not been carried beyond the $66\frac{2}{3}$ per cent intensity difference. The reasons why is not justifiable to carry the curve beyond this point are as follows. In its normal method of locomotion the blowfly larva extends its anterior end alternately to right and left, fixing its maxillal hooks in the substratum and pulling its body up by the aid of their attachment. (See also, 7, p. 176). The extent of the side-to-side swinging of the head in this process is variable and very difficult to estimate accurately.

I should place the average swinging of the larva when not subjected to sudden change of illumination as approximately 30° to 40° on either side of the mid-position. The effect of this swinging on the amount of light intercepted by sensitive surfaces inclined toward each other at an angle of 80° is indicated in figure 7. The line AB is drawn perpendicular to the line connecting the sources of light, 1 L and 3 L. The line OC is constructed at an angle to AB (31°) such that the amount of light reaching the opposite surfaces of the 80° angle, edf, constructed about OC as a bisector shall be equal when the lights are of a three to one intensity ratio. The broken lines mx and hx are constructed at an angle of 35° to OC. They represent the axial position of the anterior end of the larva at the extreme of its swing toward either side, while the angles ghi and lmn represent the resulting positions of the sensitive surfaces. In the axial position, mx, the sensitive surface mn is shaded from the light 3 L and exposed to the light 1 L. When the angle of orientation (BOC) is not great enough for the side-to-side swingings to shade one sensitive area, they would not appreciably influence orientation, because the swings being equal in extent toward either side, the total effect of the light would be approximately equivalent to its effect in the average position ($< f\,d\,e$ fig. 7). The particular angle of orientation here illustrated lies at about the critical point where the side-to-side swinging begins to alter the simple action of the lights which holds at smaller angles of orientation. Although in this case the extent of the arc of swinging in which the effect of the shading is felt is so small that it has little influence on the angle of orientation, yet at the wider angles of orientation the effect of the shading becomes rapidly greater, causing the slope of the curve to change markedly

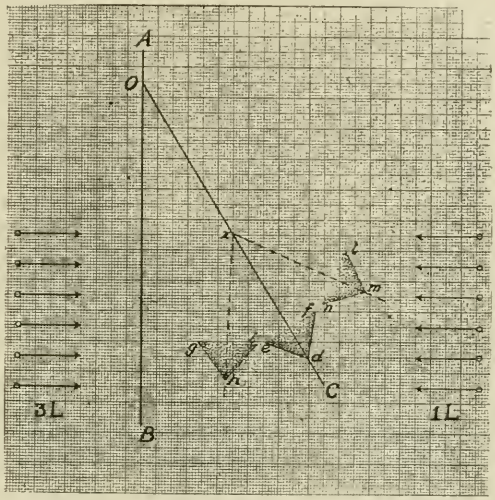

Fig. 7. For explanation see text, pages 334 and 335.

in the region where this second factor first becomes operative. (See fig. 6, experimental curve.)

Calculations have been made for the upper portion of the reaction graph on the basis of the light energy intercepted by both surfaces in the complete arc of swinging. This treatment, however, is of too uncertain a nature to be of any quantitative value. Still it yields results which, in a general way, accord with the more abrupt rise in the upper portion of the experimental curve.

We would seem, therefore, to be justified in stating that an animal possessing the peculiarities of locomotion characteristic of the blowfly larva, together with bilaterally located photosensitive surfaces inclined toward each other at an angle of 80°, which influence the musculature of the opposite sides in proportion to the stimulation they receive, would, if subjected to the conditions of these experiments, yield a type of reaction graph similar to that plotted from the measurements made on the blowfly larva.

The type of curve which would be produced under the conditions of the second set of experiments (see pp. 326–330) by a mechanism of the nature postulated is much more readily ascertainable. It will be recalled that the stimulus there employed was "a graded series of absolute intensities of opposed lights having their relative intensity maintained constant." Suppose that under an intensity ratio of three to one between the opposed lights, the axial position of the animal is changed until it attains an angle of orientation such that the amount of light falling on the opposite sensitive surfaces is equal. The position of the photoreceptive system would be that illustrated by the angle *e d f* in figure 7. As already pointed out, this equalization depends on the fact that the sensitive surface toward the light of three units intensity, (3 *L*) presents to the rays an area of one-third that presented by the symmetrical sensitive surface of the opposite side of the animal to the light of one unit intensity (1 *L*). Whatever change is made in the absolute intensities of the opposed lights, as long as this ratio is maintained, *the same axial position will equalize the stimulus bilaterally.*

We should obtain, therefore, for any intensities of fixed ratio to each other, horizontal, rectilinear reaction curves. The positions of the graphs for the conditions of these experiments were determined theoretically when the angles of orientation which would equalize the light at given intensity ratios were computed (see fig. 6 and p. 334). We therefore know the contour and position of the reaction graphs which would be produced under these conditions by the action of the mechan-

ism postulated. These curves plotted for comparison with the experimental curves of figure 5, fall in the position of the solid, horizontal lines.

By computing the average variability of the experimental data and comparing it with the values of the fluctuation of the curves, the conclusion was reached (p. 330) "that the significance of each curve is a horizontal line represented by the 'mean line' computed from the five points of a curve." The close conformity of the experimental and the theoretical curves is so striking that it needs no further emphasis.

Conclusions from such conformity as has been demonstrated above, must be drawn with extreme caution. The fact that a mechanism such as postulated would produce both types of curves obtained experimentally is in no wise to be regarded as proof of the existence of precisely such a mechanism in the blowfly larva. It does, however, present a hypothesis on which further work may profitably be undertaken. Moreover such a hypothesis offers a logical explanation of the contour of a group of stimulus-reaction graphs for which there is no other explanation available.

The most interesting phase of the results as far as the Weber-Fechner Law is concerned is the conformity with its theoretic expectation shown by the curves of figure 5. This agreement might easily have led to entire misinterpretation of the results, had not a second set of measurements been made which was clearly unconformable with the law. As both series of data were obtained under identical experimental conditions the conclusion is unavoidable that the conformity with Weber's Law, where it does occur, is entirely accidental. This does not necessarily signify that a following of the Weber-Fechner Law could not be demonstrated in the photic reactions of the blowfly larva, but that the nature of the reaction mechanism involved in the response to opposed lights is such that it dominates the other factors involved in the reaction. It is entirely possible that experiments made in such a way as to exclude the action of this mechanism for obtaining a bilateral balance of stimulation, might yield results in accord with the Weber-Fechner Curve.

VI. SUMMARY AND CONCLUSIONS

We have in this paper dealt with experiments devised for measuring the reactions of the blowfly larva so that they might be employed for comparison with various values of the acting stimulus. The measurements were so made and collected that they could be handled from

the same point of view as the available data on the comparison of sensation value with stimulus intensity. Analysis of the results obtained, with reference to the Weber-Fechner Law, brought out the following conclusions:

1. The graph expressing the values of the angular deflections of the blowfly larva from an original path of locomotion at right angles to the line connecting the sources of light, produced by a graded series of percentage differences of intensity between opposed lights cannot be regarded as conforming to the Weber-Fechner Curve.

2. Graphs expressing the values of the angular deflections produced by a graded series of absolute intensities of opposed lights having their relative intensity maintained constant, may be regarded as in conformity with the Weber-Fechner expectation.

3. The fact that two sets of data derived from the same animals, by identical experimental procedure, yield opposite conclusions so far as the Weber-Fechner Law is concerned, indicates that the agreement with the law, where it does occur, is a purely fortuitous one, and that the reactions of the blowfly larva to opposed lights are controlled by other factors.

4. It is possible to demonstrate geometrically that a photoreceptive mechanism operating after the manner of bilaterally located sensitive surfaces inclined at an angle to each other and affecting the opposite sides of the musculature in proportion to the stimulus they receive, would determine *both* types of curves which have been plotted from experimental measurements of the photic reactions of the blowfly larva.

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THE INFLUENCE OF THE EXTRACT OF THE POSTERIOR LOBE OF THE HYPOPHYSIS UPON THE SECRETION OF SALIVA

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Of the glands of internal secretion, probably one of the least understood, though one of the more important is the hypophysis, least understood, because of its inaccessibility and its seeming paradox in that the anterior lobe is the vital portion, though its extract is physiologically inactive, while the posterior lobe, although not essential to life, furnishes an extract with marked physiological properties; important because of the changes associated with its pathology. The action of the extract of the posterior portion of the pituitary body on the circulatory system has been fairly well established, but its effect on secretion is still in dispute. Schäfer and Herring (1) concluded that it has a specific action on the renal epithelium, while Houghton and Merrill (2) maintained that the diuresis observed is due to vascular changes. Hallion and Carrion (3) noted a vasodilation following the primary vasoconstriction of the kidney, and also that the diuresis followed this vasodilation. Their results were verified by King and Stoland (4) who were unable to obtain diuresis except during vasodilation in the kidney. Frankl-Hochwart and Frölich (5) asserted that pituitary extract has no influence on the chorda tympani nerve nor on any of the secretory nerves to the salivary glands. Pemberton and Sweet (6) found that pituitary extract inhibits the secretion of pancreatic juice, supposedly by action on the cells. Edwards (6) maintains that no inhibition occurs except what can be accounted for by the vasoconstriction. In view of these results it would seem that ample opportunity was offered for further work, and at the suggestion of our instructor, Dr. O. O. Stoland, we decided to conduct a series of experiments to determine, if possible, the effect of pituitrin upon the secretion of saliva, and whether this effect is due to vascular changes in the organ or to a specific action on the cells or the secretory nerves.

On account of its accessibility and the fact that it has definite secretory and vasodilator fibers from the chorda tympani and vasoconstrictor and secretory fibers from the sympathetic, the submaxillary gland seemed to lend itself more readily than any other organ to our work. It appeared to us that the results obtained with this gland could be more readily controlled and checked. The work done by Mathews (7) on the effect of shutting off the blood supply to the submaxillary gland was of prime importance to us, in that his results show that the function of the gland was not impaired by shutting off the blood supply, but that with restoration of circulation the flow of saliva returned. He also showed that saliva would flow for almost a minute after the blood supply was shut off. From this we may conclude that a mere decrease in blood supply would not be sufficient to cause an almost immediate cessation of salivary flow.

The experiments reported in this paper were carried out on healthy, well-nourished dogs, varying from 4 to 20 kilos in weight. The animals had no special preparation except that they were not fed on the day of experimentation. Ether was used as the anaesthetic throughout and was so administered by a tracheal cannula that a relatively constant degree of anaesthesia was attained by the time injections were to be made. Wharton's duct was isolated, usually on the left side, and a glass cannula inserted. The blood pressure changes were recorded by a mercury manometer connected with the right carotid artery. The external jugular vein and its branches were dissected out to expose the venous return from the gland. In most cases this consisted of a branch from the upper and anterior portion of the gland to the external jugular just before it passes through the capsule of the parotid gland and a branch from the posterior portion of the gland to the external jugular or to a branch of the latter. Variations consisted in having only one of these, most commonly the anterior branch. All branches of the external jugular except those coming from the gland were ligated, and a cannula, well paraffined and oiled, was inserted into the external jugular vein, and the blood flow through the gland recorded by the drop method. The flow of saliva from the gland was recorded by the same method. All injections were made into the femoral vein towards the heart. Continuous tracings were taken of the blood flow and salivary flow from the gland and the general blood pressure except in the first few experiments where the flow of saliva and blood pressure alone were recorded. These experiments were performed as a control against later experiments where longer

exposure of the gland was necessary. We found that exposure did not alter the results. The pituitrin made by Parke, Davis and Company was used exclusively in our work. After the animal was ready for injection, we usually found that the flow of saliva was very meager, and in order to stimulate a flow one of two methods were resorted to: the chorda tympani nerve was stimulated peripherally by a weak faradic current, or the flow was augmented by the injection of small amounts of pilocarpine. When pilocarpine was used, pituitrin was not, as a rule, injected until the blood pressure had again returned to normal. In order to control our results, variations in procedure were frequent, and the results will be given in the order in which the experiments were performed, as far as possible. The same procedure was followed until consistent results had been obtained. One cubic centimeter of pituitrin was injected unless otherwise stated.

Throughout all our work on dogs one fact, about which there could be no reasonable doubt or question because there was no exception, stood out prominently, namely, that the intravenous injection of pituitary extract invariably caused a marked decrease in the flow of saliva from the submaxillary gland. From all our experiments we found that the average slowing of saliva due to the action of pituitrin was 82.4 per cent in 47 injections, and that of these in only 13 was there a slowing of less than 75 per cent. In many cases the flow of saliva ceased altogether for a short time. In some animals more than one injection of pituitrin was made, but we failed to find in any of these cases that repeated injections decreased its effect at all except in one experiment where a large cat was used: In this case we found that the slowing of saliva was decreased after the second injection of pituitrin, and that there was hardly any slowing after the third. In all our work, also, an accompanying diminution of the blood flow from the gland was noted following every injection of pituitrin. Now the question arose, was this great decrease in salivary flow due to changes in blood supply to the gland, associated with the vasoconstriction caused by pituitrin, or to its effect upon the secretory nerves or gland cells of the organ, or to a combination of both of these? In order to analyse the causes of this great diminution in salivary secretion, we averaged the results obtained on the injection of pituitary extract during the stimulation of the chorda tympani nerve, during the action of pilocarpine, during the action of adrenalin, and during the action of chrysotoxin. The results are recorded in Table 1.

A series of three experiments was performed where the injection

TABLE 1

TREATMENT BEFORE AND DURING THE PITUITRIN ACTION	NO. OF ANIMALS	BEFORE THE INJECTION OF PITUITRIN		AFTER THE INJECTION OF 1 CC. OF PITUITRIN		
		Blood (drops per min.)	Saliva (drops per min.)	No. of injections	Blood	Saliva
Faradization of chorda tympani	3	84	21	6	30.5	4.6
Pilocarpine stimulation	13	58.6	13.7	16	16	2.57
Adrenalin stimulation	4	21.6	5.3	4	9.8	1.5
Action of chrysotoxin	7	48.2	5	7	15.5	0.35

of pituitrin was made during the faradization of the chorda tympani. Here it was observed that the slowing of saliva was 80 per cent in six injections, while the blood flow decreased only 64 per cent. We found that in most cases the blood flow almost stopped momentarily, but later the flow increased greatly while the salivary flow continued decreasing (see fig. 1). This would indicate that pituitrin acts upon

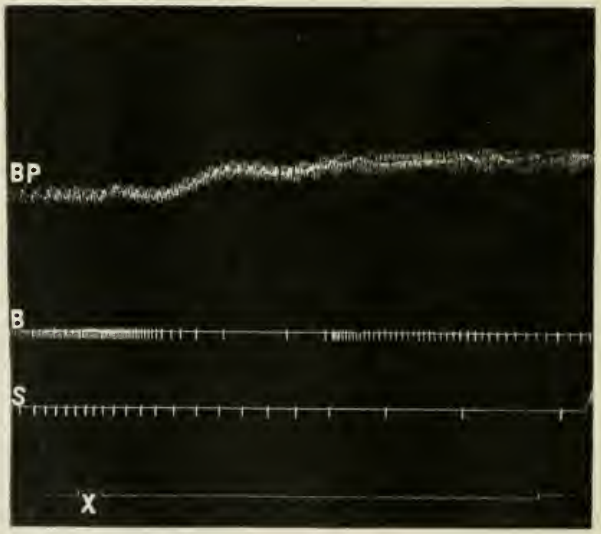

Fig. 1. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during light faradization of the chorda tympani. *BP*, blood pressure; *B*, drops of blood from the venous return of the submaxillary gland; *S*, saliva from Wharton's duct in drops; *X*, 1 cc. of pituitrin injected.

the secretory mechanism of the gland by some other method than by vasoconstriction alone, probably by blocking or decreasing the irritability of the secretory fibers, for, as was noted above, the salivary secretion slowed even when the blood flow was fairly rapid.

We observed that during pilocarpine stimulation the slowing of the salivary flow, following the injection of pituitrin, was equal to that obtained during chorda stimulation, but that while the decrease in the blood flow during pilocarpine action was 73 per cent (see fig. 2), that during faradization of the chorda was 64 per cent. As is well known, pilocarpine stimulates the endings of the secretory nerves, while excitation of the chorda tympani affects both the vasodilator

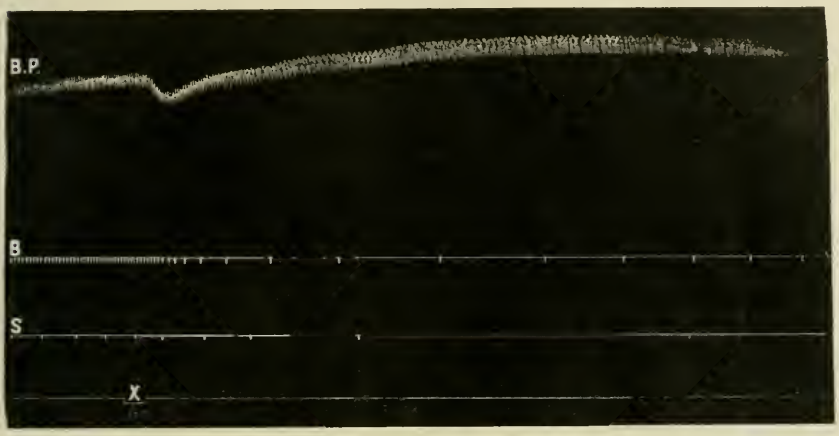

Fig. 2. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during the action of pilocarpine. The letters are same as in figure 1.

and secretory nerves, and hence the decrease in blood flow, due to the action of pituitrin, would not be so marked in the latter case. Some increase in blood flow was noted on stimulation with pilocarpine, but was evidently the result of the greater activity of the gland rather than to any action of this drug upon the vasodilators. From this we may assume that pilocarpine acts in the same way upon the submaxillary gland as does faradization of the chorda, with the added advantage that in the former case the vasodilators are probably not affected. Now the fact that the slowing of saliva following the action of pituitrin was the same under two different kinds of stimulation, both involving the secretory fibers, coupled with the fact that the accompanying

decrease in venous return was not the same because the vasodilators were probably not stimulated to the same extent in one case, would seem to indicate that this drug inhibits in some way the secretory fibers to the gland.

Another result obtained with pilocarpine, seemingly the opposite of the normal, was the great rise in blood pressure following the injection of this drug during the action of pituitrin. Since pilocarpine normally causes a slowing of the heart and a fall in blood pressure, we are at a loss for a satisfactory explanation of this apparent paradox. The heart beat was slowed, but its amplitude was in some cases markedly increased.

TABLE 2

The results recorded in this table show the effect of pilocarpine upon the flow of blood and saliva before and during the action of pituitrin and the effect of adrenalin upon the same under like conditions.

INJECTION	NO. OF ANIMALS	NO. OF INJECTIONS	BLOOD (DROPS PER MIN.)	SALIVA (DROPS PER MIN.)
Pilocarpine	5	7	59.5	24
Pituitrin	5	7	15.4	2.1
Pilocarpine during action of pituitrin	5	7	20.5	3.2
Before adrenalin	4	4	31	2
Adrenalin	4	4	49	5.5
Pituitrin before injection of adrenalin	7	8	12	2.8
Adrenalin following injection of pitruitrin	7	8	17.7	1.5

We found further that pilocarpine was relatively inactive during the action of pituitrin. In this series of experiments (see Table 2) an injection of pilocarpine was made to stimulate a rapid flow of saliva, followed by an injection of 1 cc. of pituitrin, and at various stages of pituitrin action a second injection of pilocarpine was made. Table 2 shows that although the second injection of pilocarpine causes a slight increase in blood and salivary flow during the action of pituitrin, it is relatively inactive, because the increase can be accounted for by the partial recovery from the action of the pituitrin. In some of our experiments it was noticed that there was no increase in the flow of saliva, even though the blood flow increased a little. This would indicate that pilocarpine may have some action on the vasodilators, or more

likely that the activity of the gland was increased without apparent increase in the salivary flow, possibly by increasing the organic constituents. Since we made no quantitative determination of organic matter in the saliva, we are unable to do more than merely to state this possible explanation. In one experiment, where two successive injections of pituitrin were made to stop the flow of saliva, the real effect of pituitrin was probably shown better than in any other of this series. During this action of the hypophyseal extract it was observed that though the flow of blood was doubled after the first injection of pilocarpine, the flow of saliva remained the same, and when the second injection of pilocarpine was made eight minutes later, even then the saliva increased in less measure than the flow of blood, which is contrary to the normal action of pilocarpine. At this time the action of pituitrin should have been fairly well spent, for the blood pressure had almost returned to normal. The above observations would seem to show that the action of pituitrin is partly, at any rate, upon the secretory fibers to the gland.

In a series of four experiments we attempted to show the effect of shutting off the blood supply on the flow of saliva normally and after the injection of pituitrin. Normally the salivary flow ceased in less than one minute after shutting off the blood supply, and the same result was obtained after injection of pituitrin, the pituitrin being injected half a minute before the blood supply was shut off. This procedure failed to give any definite results. The only effect noted was that the blood flow returned slightly more slowly, and the saliva flow did not return for one minute, while normally both returned together. This is borne out by the work of Mathews (7) who shut off the blood supply for long periods of time (7 to 30 minutes).

In our experiments the blood supply was shut off for one minute In one case the pituitrin was injected while the blood supply was shut off for 1.25 minutes and when the circulation was restored, the flow of saliva and blood both returned immediately but later slowed due to pituitrin in the blood. This is of interest in so far that it shows the peripheral action of pituitrin, because the nervous mechanism of the gland was intact. Aside from this, shutting off the blood supply furnished no other evidence than that we had already obtained by other methods, and therefore it was abandoned.

Comparing the average percentage of slowing of blood and saliva in 33 injections of pituitrin (see Table 1), we noted that the flow of saliva decreased 81.8 per cent, while the slowing of blood was 65 per

cent. This in itself would point to the probability that the decrease is not due entirely to vasoconstriction but to some other factor also. Can it be attributed to the inhibitory effect on the secretory fibers or to the toxic effect on the gland? The latter would be ruled out by the general lack of toxicity of pituitrin. This leaves us the hypothesis that the inhibition of secretion is practically due to the action on the secretory fibers to the gland, especially in view of the ineffectiveness of pilocarpine during the action of pituitrin.

In order to obtain further evidence as to the influence of pituitrin upon secretion, it was concluded to try the effect of epinephrin, the great general vasoconstrictor, which acts on the endings of the vasomotor nerves. However, we found that this drug invariably caused a vasodilation in the submaxillary gland, while effecting a general vasoconstriction. Epinephrin then presumably stimulates the vasodilator endings in this gland. Epinephrin also increased the flow of saliva; in some cases we used it to start the secretion (see Table 1). In four injections where adrenalin was used to initiate the secretion, the salivary flow decreased 72 per cent, while the blood flow decreased 55 per cent after the injection of pituitrin, thus practically paralleling the results obtained during faradization of the chorda tympani (see Table 1), a fact which would support the hypothesis that pituitrin acts upon the secretory fibers.

From Table 2, we note that when epinephrin was injected during the action of pituitrin, it caused an increase in the flow of blood, but a decrease in the flow of saliva (see fig. 3). This diminution in salivary secretion during vasodilation in the gland must be due to the increased amount of pituitrinized blood passing through the gland on account of the action of epinephrin, thus augmenting the effect on the secretory fibers. As far as we can see, the above evidence alone would go far to prove our case.

In the hope of abolishing or decreasing the action of the vasoconstrictors to the gland, we made injections of Jacobi's chrysotoxin (8) and followed this by injections of epinephrin and pituitrin. This chrysotoxin was made by extracting powdered ergot with ether, evaporating off the ether, and precipitating the oily extract with light petroleum. The precipitate was dissolved in physiological salt solution and then injected. No effect was noted on blood pressure or salivary flow, but with one preparation of chrysotoxin especially we found a marked increase in the amount of venous return from the gland, which may be attributed to the depression of the tone of the vasoconstrictors

to the gland. The injection of adrenalin during the action of this ergot preparation caused only a slight primary rise in blood pressure, followed by a fall, or in some cases there was no change at all in the blood pressure noted; in one case we got "vasomotor reversal." The flow of blood and saliva after adrenalin was unaffected to any marked degree, though in some instances a slight slowing of both was observed during the primary rise.

A further study with the method of preparation and properties of the ergot preparations since the completion of our experiments has led us to believe that the material used in our work was somewhat lacking in strength and purity; its action, however, was sufficient for our in-

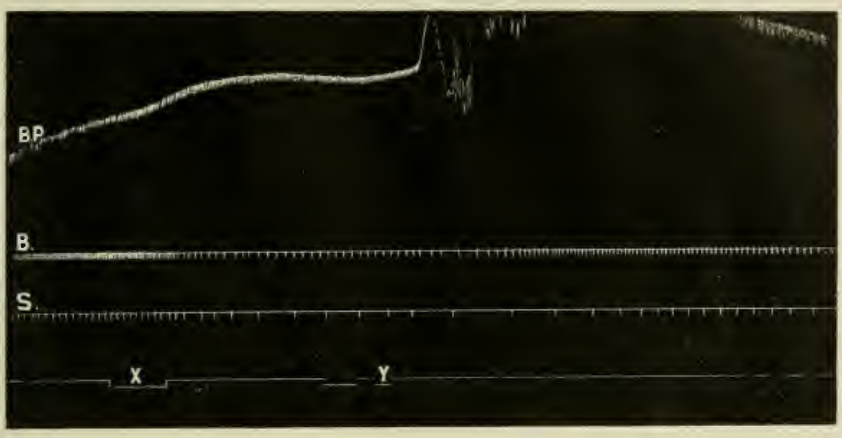

Fig. 3. The effect of epinephrin upon the flow of saliva and blood during the action of pituitrin. The letters are same as in figure 1; *Y*, injection of 2 cc. 1–50,000 epinephrin.

tentions. Having found that chrysotoxin inhibited the action of the vascoconstrictors to a marked degree, we were ready to try the effect of pituitrin during the action of chrysotoxin. We found that normally the vasoconstriction after injection of pituitrin was almost invariably preceded by at least a slight fall. When pituitrin was injected during the action of chrysotoxin we observed that the primary fall in blood pressure was, as a rule, greater than normal, while the secondary rise, though characteristic, was in most cases less marked. It was noted that the flow of saliva diminished before the decrease in blood flow was noticeable. In all but two cases the salivary slowing was noted while there was an increased output of blood from the gland (fig. 4). Dur-

ing the action of this drug the vasoconstriction after pituitrin was much less marked in most cases, the blood flowing fairly rapidly, while the decrease in flow of saliva was as great as or greater than that obtained in our other experiments.

The results obtained with chrysotoxin constitute our strongest evidence that pituitrin acts by inhibiting the secretory fibers. A number of workers have attributed the vasoconstriction caused by pituitrin to a direct action on the muscles of the arterioles; our experiments with chrysotoxin would indicate that whatever the true seat

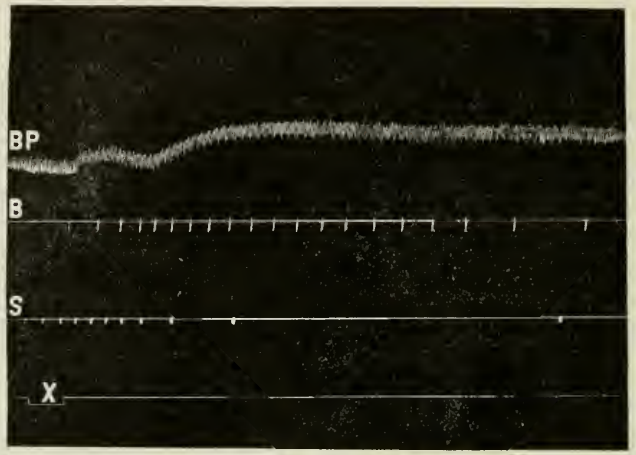

Fig. 4. The effect of pituitrin upon the flow of saliva and blood from the submaxillary gland during the action of chrysotoxin. The letters are same as in figure 1. The chrysotoxin was injected about 2 minutes before the injection of pituitrin and the dose had previously been shown to be sufficient to depress the irritability of the vasoconstrictors.

of action may be, this substance depresses the sensibility of the mechanism affected by pituitrin. Pituitrin probably acts upon the endings of the vasomotor nerves as well as upon the involuntary muscles.

SUMMARY AND CONCLUSION

1. Pituitrin invariably caused a diminution in flow of blood and saliva from the submaxillary gland, as shown from results obtained from 30 dogs and 1 cat.

2. The decrease in flow of saliva was greater than the accompanying decrease in blood flow.

3. The slowing of blood was less marked if the injection was made during faradization of the chorda tympani than during pilocarpine-stimulation, while the slowing of saliva was the same.

4. Pilocarpine was relatively ineffective even when injected seven or eight minutes after pituitrin.

5. While epinephrin normally caused a vasodilation of the gland and increase in salivary secretion, epinephrin during the action of pituitrin had the normal effect on the blood flow but caused a diminution in salivary flow, probably due to the greater quantity of pituitrin coming in contact with the gland.

6. When pituitrin was injected during the action of chrysotoxin, the decrease in the flow of saliva set in before the vasoconstriction in the gland occurred. In five out of seven cases, the flow of saliva slowed while there was active vasodilation in the gland.

From these results we must conclude that the decrease in flow of saliva following the injection of pituitrin is due to inhibition of the action of the secretory nerves to the submaxillary gland, but also due in part to the accompanying vasoconstriction, which is caused by direct action on the muscles of the arterioles or the effect on the peripheral endings of the vasomotor nerves, but more probably to the effect on both. The decrease in output of blood from the gland may be also due to the decreased activity of the gland.

We wish to express our thanks to Dr. O. O. Stoland for his valuable suggestions and criticisms in performing this series of experiments.

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THE INFLUENCE OF THE VAGUS NERVE ON THE GASEOUS METABOLISM OF THE KIDNEY

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Asher and Pearce (1) have reported a series of experiments the results of which they interpret as demonstrating the presence of renal secretory fibers in the vagus nerve. In these experiments, which were done on decerebrate animals, the urine obtained during a period of vagus stimulation exceeded in amount that obtained during a normal period of equal duration. The technical difficulties of the operation made it impossible to carry out more than a few successful experiments, so that a relatively small number of results are reported.

To secure additional data in this connection is the object of the present research, and for this purpose we have not depended on the rate of urine formation, but have sought for evidence of changes in the functional activities of the renal cells. To do this we have taken advantage of the fact that every increase in tissue activity is accompanied or followed by an increase in oxygen consumption. Barcroft (2) and his co-workers have established such a relation in the case of the kidney, their researches having shown that two types of diuretics may be distinguished: one whose diuretic action is accompanied and the other unaccompanied by an increase of oxygen consumption. It may be considered that the action of the former type of diuretics (urea, caffein and sodium sulphate), is due primarily to an actual stimulation of the excreting cells of the kidney, while the action of the latter type (sodium chloride, etc.) depends on an alteration in the osmotic pressure of the blood, produced by the injection of these salts into the body. This relationship being established, it follows that an estimation of the oxygen absorption of the kidney will be an index of the activity of the renal cells under varying conditions.

The diuresis produced by vagus stimulation must be a result of increased renal activity and for two reasons: viz., 1st, it is impossible for a nerve to change directly the osmotic pressure of the blood; 2nd,

the blood flow through the kidney does not become altered under the conditions of the above described experiment (3).

An increase in oxygen consumption by the kidney should accompany the stimulation of the vagus if this nerve really contains renal secretory fibers. To test this an estimation was made of the oxygen intake of the kidney under the conditions of Asher and Pearce's experiment; that is to say, the percentage difference between the oxygen af the arterial and venous bloods was compared. The reflex disturbances arising from the manipulation of the kidney or ureters is discounted by comparing the changes occurring in the stimulated kidney with those obtained in the opposite kidney, which was previously denervated. The fact that the vagus does not influence the circulation of the kidney (3) makes the estimation of the volumes of the blood flow unnecessary in measuring the oxygen consumption, and makes it possible to determine this by comparing the percentage amounts of oxygen in the arterial and venous bloods before and during vagus stimulation.

METHODS

The preparation of the animal conformed in all essentials to that followed by Asher and Pearce. Dogs were etherized and quickly decerebrated. The vasomotor control exercised on the renal blood vessels through the splanchnic nerves was removed on the left side by cutting the nerve just above the adrenal gland. The kidney on the opposite side (right) was at the same time completely removed from the nervous system by severing the nerves surrounding the renal vessels and searing the vessels with concentrated phenol solution. Electrodes were placed on the vagi below the level of the heart, and the nerves cut in the neck. The viscera were retracted and held in such a manner as to expose the renal veins on both sides. Samples of 1 cc. each of blood were removed from the renal veins and from the carotid artery through fine needles into hypodermic syringes containing $\frac{2}{10}$ cc. of hirudin dissolved in boiled Ringer's solution. After removing the blood, the syringe was sealed by sucking up a small quantity of mercury and capping the end, from which the needle had been detached, with a closed rubber tube. The oxygen in the various samples was estimated by the so-called differential method as described by Barcroft for determining the total oxygen in $\frac{1}{10}$ cc. of unsaturated blood (4). Artificial respiration was maintained throughout the experiments and every precaution was taken to keep the blood pressure at a constant level.

In most of the experiments this was accomplished fairly well. Twenty-five to thirty minutes elapsed between the decerebration and the taking of the blood samples. The possible disturbing influence of anaesthesia was thus eliminated.

The procedure followed in obtaining the samples of blood was the same in all the experiments. The first samples of blood were removed from the renal veins of both kidneys and from the carotid artery, and were used to determine the normal gas exchange. Immediately afterwards the vagi were stimulated with a current of approximately the same strength as that used by Asher and Pearce, for alternating periods of one minute with one minute intervals between. During the fourth minute of stimulation the second samples of blood were removed as above described and were used to determine the exchange occurring during stimulation.

The oxygen percentages of the venous bloods were compared with those of the arterial bloods of the corresponding periods, and the number of cubic centimeters of oxygen absorbed per cubic centimeter of blood calculated in each case. The right kidney, since its nerves had been destroyed, could not be affected directly by stimulation of the vagus nerve. Its oxygen consumption should therefore be constant, or if any change was found in its oxygen intake, it must be sought in some systemic condition which would effect the left kidney in the same degree.

EXPERIMENTAL RESULTS

In the accompanying table the results of the last four experiments are tabulated. The figures in the third column give the oxygen content of the arterial blood, and those in the fourth and fifth columns the oxygen content of the venous blood of either kidney before and during a period of vagus stimulation. The sixth and seventh columns give the actual cubic centimeter of oxygen taken from 1 cc. of blood by either kidney, and the last two columns, the percentile change in oxygen consumption which accompanies the stimulation of the vagus.

Experiment No. 5 is the only one of the series in which there is a greater amount of oxygen used during a period of stimulation of the vagus than can be attributed to systemic conditions as reflected in the secretion of the right denervated kidney. In all of the remaining experiments the change in oxygen consumption in the left kidney accompany-

ing vagus stimulation is less than that found in the case of the denervated right kidney.

The operative procedure of these experiments, as in those of Asher and Pearce, is very severe, and it is difficult to maintain the animal in an unchanging condition in regard to the circulation, respiration and renal function. The tables show that in spite of pains taken to maintain the normal respiratory function, there was a marked decrease in the oxygen content of the arterial blood during the stimulation of the vagus. The exact cause of this change is not at present clear. The actual oxygen consumed by each kidney, however, remained practically constant, as shown by the figures in columns six and seven of the table.

In the experiments reported by Barcroft and Brodie, in which the effects of certain diuretics on the urine flow and oxygen consumption of the kidney were investigated, an increase in urine excretion was accompanied by a like change in oxygen used by the kidney. These investigators were unable to estimate the actual amount of oxygen necessary for the excretion of a fixed amount of urine, but the experiments show that it is of such magnitude that the oxygen intake can be made a test of the physiological activity of the kidney.

The failure to obtain evidence of an increased gaseous exchange following stimulation of the vagus nerve under the conditions reputed to produce a diuresis, as in the above series of experiments, can be interpreted by assuming, either that the diuretic effect failed to appear, or that it occurred independently of any energy change in the kidney. It is to be regretted that the experimental procedure is such that simultaneous investigations of the gaseous exchange and urinary outflow are not practicable. It is, however, reasonable to suppose that in a series of eight experiments, some would show a change in oxygen consumption if the vagus nerve exerts any marked secretory influence on the kidney.

SUMMARY

The oxygen consumption of the left kidney before and during the stimulation of the vagus below the level of the heart and after section of the left splanchnic nerve, remains unchanged. This offers evidence against the supposed existence of renal secretory fibers in the vagus nerve.

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TABLE 1

EXPERIMENT PERIOD		CC. OF OXYGEN PER 1 CC. OF BLOOD			CC. OF OXYGEN CONSUMPTION PER 1 CC. OF BLOOD		PERCENTILE CHANGE IN OXYGEN CONSUMPTION DURING PERIOD OF VAGUS STIM.		REMARKS
		Carotid blood	Right kidney (denervated)	Left kidney (experimental)	Right kidney	Left kidney	Right kidney	Left kidney	
5 April 8	Normal	0.2035	0.1650	0.1650	0.0385	0.0385	+ 7	+27	Blood pressure: 135 mm. Hg. throughout experiment
	Vagus stimulation	0.1847	0.1430	0.1320	0.0417	0.0495			
6 April 17	Normal	0.2035	0.1595	0.1650	0.044	0.0385	+25	+13	Blood pressure: 85 mm. at beginning and 75 mm. Hg. at end of experiment.
	Vagus stimulation	0.1650	0.1100	0.1211	0.055	0.0440			
7 May 6	Normal	0.2090	0.1705	0.1540	0.0385	0.0550	±00	−12	Blood pressure: 80 mm.
	Vagus stimulation	0.1705	0.1320	0.1210	0.0385	0.0490			
8 May 21	Normal	0.1420	0.1155	0.0990	0.0165	0.0330	±00	−80	Blood pressure: 80 mm. at beginning of experiment. During the time of taking sample of blood at time of vagus stimulation, the blood pressure rose 10 mm.
	Vagus stimulation	0.1155	0.0990	0.1100	0.0165	0.0050			

CHANGES IN IODINE CONTENT OF THE THYROID GLAND FOLLOWING CHANGES IN THE BLOOD FLOW THROUGH THE GLAND

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This work was undertaken at the suggestion of Dr. Carlson, primarily to determine the influence of changes in the circulation of the thyroid gland on the iodine content of the gland. This point does not seem to have been controlled in the recent interesting work of Rahe, Rogers, Fawcett and Beebe, (1) on the effect of stimulation of the thyroid nerves on the iodine content of the gland. It seems clear that the data reported by Rahe, et al, cannot be construed as proving the presence of secretory nerves to the thyroids, until it is shown that the vaso-motor changes in the thyroid induced by stimulation of the thyroid nerves are not by themselves capable of inducing the changes in the iodine content.

II. LITERATURE

According to Rhinehart (2) the nerve supply to the thyroid is made up of filaments from the cervical sympathetic, entering the gland with the perivascular connective tissue and tunica adventitia of the thyroid arteries. In man and the cat the filaments have been demonstrated to arise only from the superior cervical ganglion of the sympathetic. In the dog some observers claim that nerve filaments from the vagus also enter the thyroids. This is denied by Rhinehart. In the thyroid glands the nerves form elaborate perivascular nerve plexuses and end, at least in part, in the walls of the blood vessels.

Ossakin (3) gives a brief review of the literature on the vaso-motor and possible secretory function of the thyroid nerves. Brian stimulated the vago-sympathetic and inferior laryngeal nerves and studied the histological and vaso-motor changes produced in the thyroids. He concluded that these nerves carry both secretory and vaso-motor fibers. The vaso-motor function has been described as the sole function of the

thyroid nerves by Sacerdatti. V. Cyon reports experiments showing on stimulation of the nervus thyroideus superior, a slight diminution in the carotid blood pressure with a change in the volume of the gland. By measuring the blood pressure in the arteria thyroidea superior V. Cyon concluded that both vaso-constrictors and vaso-dilators are contained in the laryngeal nerves, the constrictors predominating in the superior.

Morat and Sinakewitsch by plethysmography showed that stimulation of the cervical sympathetic gives a pressor effect—a decrease of the amount of blood in the thyroid gland. The latter investigator showed that on extirpation of the superior cervical ganglion or paralysis of this ganglion with nicotine no effect on the thyroid was obtained on stimulation of the sympathetic. From this he concluded that the sympathetic fibers are synapsed in the superior cervical ganglion before reaching the thyroid gland.

Katzenstein reports marked degenerative changes in the thyroid on cutting the thyroid nerves, and concludes that the nerves contain true sensory fibers. These degenerative changes on cutting the nerves are attributed solely to changes in the blood supply by Luebeke.

Wyss and Andersson failed to obtain histological change in the thyroid following injection of pilocarpine.

Huerthle stained the thyroid gland after stimulation of the laryngeal nerves and found no change. He concludes, therefore, that these nerves are not secretory but vaso-motor.

Martini by stimulating and Horsley by cutting the thyroid nerves report no change in the histology of the gland.

Wiener cut the vago-sympathetic and paralyzed the superior cervical ganglion reporting no histological change in the thyreoglobulin content of the gland. But when the inferior cervical ganglion was removed leaving the superior intact he reports a decrease in the weight of the gland and decrease in the thyreoglobulin content, from which he concludes that the thyroid nerves have both a trophic and secretory function.

Asher and Flack (4) working on rabbits, cats and dogs compared excitability of the depressor nerve before and after stimulation of the thyroid nerves, and also the effect of a small intravenous injection of adrenalin before and after such stimulation. They conclude that under exactly similar experimental conditions the excitability of the depressor nerve is increased during or immediately following the stimulation of the thyroid nerves. They also report that this stimulation of the thyroid nerves sensitizes the animal to epinephrin. They as-

cribe these effects to the action of an increased quantity of thyroid secretion poured into the blood on stimulation of the thyroid nerves. Stimulation of the thyroid nerves failed to produce these effects after the extirpation of the thyroid gland. They also claim that intravenous injections of thyroid extract act similarly to stimulation of the thyroid nerves.

The work of Asher and Flack was repeated and apparently confirmed by Ossakin (3) working in Asher's laboratory.

According to the most recent work of Rahe and co-workers (1) stimulation of the nerves to one thyroid lobe decreases its iodine content as compared with that of the control lobe. They conclude that the physiologically active substance of the thyroid is discharged into the circulation on stimulation of the thyroid secretory nerves.

III. EXPERIMENTAL METHODS

All of the experimental work was done on dogs.

Analyses were made on the glands of ten normal animals to determine the relation of the iodine content in the two lobes. The glands were analyzed in duplicate, for iodine content according to Hunter's method (5), modified according to Koch (6). Part of each lobe was dried to constant weight for total solids.

Two series of stimulation experiments were run to determine whether the stimulation of the nerves leading to one lobe causes a diminution in its iodine content. The stimulation was applied to the cervical sympathetic, isolated from the vagus sheath, and to the nerve filaments accompanying the superior thyroid vessels.

The control and stimulated lobes were, in a number of cases, stained for the active secretion to determine whether the stimulation produced histological changes parallel with the chemical changes.

The vascular changes produced by stimulation of cervical sympathetic and the thyroid nerves, were studied both by the rate of the venous outflow and by the plethysmographic methods.

A series of ten animals was run in which the blood supply to one lobe was mechanically interfered with to an extent comparable to that produced on stimulation of the nerves. The main thyroid artery was cleaned of all connective tissue and accompanying nerve filaments. The blood supply was momentarily, (1–2 seconds), cut off, by compressing the artery for two seconds at ten second intervals.

Dogs with slightly enlarged thyroids were selected provided the two lobes were very nearly the same size. Having the pick of a large amount of material it was not difficult to select dogs of this type. Glands of this type were chosen in order to have a sufficient amount of material to run duplicate iodine determinations and to dry a portion for total solids.

The details of the analytical procedure are as follows: The fresh glands were carefully trimmed of all connective tissue and blood vessels and accurately weighed; they were then hashed in a lead-pan, re-weighed in the pan from which a portion was weighed into a carefully weighed glass dish to be dried for total solids, and the two other portons weighed into nickel crucibles and covered with 6 grams of pulvierized sodium hydrate and 2.6 grams of postassium nitrate. The crucibles were then placed on the steam bath for a few hours, the material going into solution in its own liquid. The crucibles were placed in the drying oven and heat applied slowly at 110°C. running up to 140° to 150°C. A little experience made it possible to remove the material at a stage at which it was easily pulverized. It was then mixed with 6.4 grams of fusion mixture and covered with 10 grams of the same, according to Hunter. From this point on the method as modified by Dr. Koch (6) was followed exactly. Care was taken to dry the material to constant weight in order to determine whether the diminution in iodine on stimulation calculated on the basis of fresh tissue is also true when calculated on the basis of dried substances.

In the stimulation experiments both lobes were left *in situ* until the end of the period of stimulation in every case. We cannot consider a lobe removed before three hours of anesthesia a control of the one removed at the end of this period since we have no knowledge of the nature or extent of the changes which may take place during this time. Light ether anesthesia was used in every case. The nerves were stimulated by brief periods of weak tetanizing current. The effectiveness of the stimulus was controlled by the effect on the pupil, the current used being 3–4 times stronger than the minimum for pupil dilatation. Gruber (7) has shown that the threshold stimulus for the vaso-constriction function of the cervical sympathetic is 6.95 B units while that for the pupil dilation is only 2.27 B units.

Dr. R. R. Bensley has devised a new stain for the thyroid secretion. Portions of several of the stimulated and control lobes were prepared in Dr. Bensley's laboratory and stained by him with a special stain for the thyroid secretion.

IV. RESULTS

Our results in the control series of normal dogs are summarized in Table I. It is evident from this table that the iodine content per gram varies widely in different individuals, but *is practically identical in the two thyroid lobes of the same animal.* The average difference between the two lobes in the case of the fresh tissue was 0.00157 per cent; in case of the dried tissue 0.01488 per cent.

According to Marine (8) the iodine content per gram is practically identical in the two thyroid lobes in dogs.

It is, therefore, evident that the per cent of iodine in one lobe of the thyroid will serve as control of the other, provided one is not dealing with diseased glands.

In the first series of stimulation experiments the cervical sympathetic was stimulated after isolation from the vagus sheath. The data are given in Table II. The average loss in per cent fresh tissue, of iodine in the stimulated lobe was 0.00794 but in Dog I, in which the per cent of iodine was highest, the loss on stimulation was 0.01499 per cent per gram of thyroid or approximately 12 per cent iodine. Dogs I–V were fed about 25 grains of potassium iodide per day for three days previous to the experiment. This was done to insure the presence of iodine in the gland and was discontinued after coming to the conclusion that with the method of Hunter iodine could be detected in every gland even though we were dealing in many cases with hyperplasia. It is interesting to note that in Dog I where the loss was greatest the stimulation period was $3\frac{3}{4}$ hours; in Dog XII where the loss was least the stimulation lasted only $1\frac{1}{2}$ hours. Dog XI did not give a decrease on stimulation, the probable explanation being that we stimulated the lobe with the greater per cent of iodine and the loss was not enough to make it less but only tending to equalize the amounts. On the whole our results show the greater decrease in the iodine content the longer the period of stimulation of the thyroid nerves.

In the second series the thyroid nerves were stimulated near their entrance into the gland with the superior thyroid artery. The blood supply was not mechanically interfered with. The results of this series are given in Table III. It will be seen that the stimulation caused a decrease in the iodine content in every case except Dog II. The average iodine loss per fresh tissue, excepting Dog II is 0.00510 per cent. The average loss calculated for dry tissue is 0.03516 per cent.

The results of these two series confirm those of Rahe, et al, but the

percentage loss is not as great as reported by them. It is apparent from Tables II and III that there is in nearly every case a greater per cent of total solids in the case of the stimulated lobe. Of course the per cent of total solids varies somewhat in the two lobes of the same animal in the normal series, (Table I), but it is obvious that we could not have chanced in every case, to stimulate the lobe with the smaller water content.

As stated before, the vascular changes, produced in the thyroid on stimulation of the thyroid nerves, were studied by the rate of venous

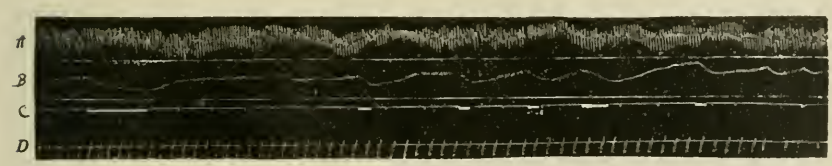

Fig. 1. Showing vaso-constriction in the thyroid gland on stimulation of the cervical sympathetic nerve. *A*, carotid (left) blood pressure. *B*, pehethysmograph record of the right thyroid lobe. *C*, showing stimulation of right cervical sympathetic nerve. *D*, time 3½ seconds.

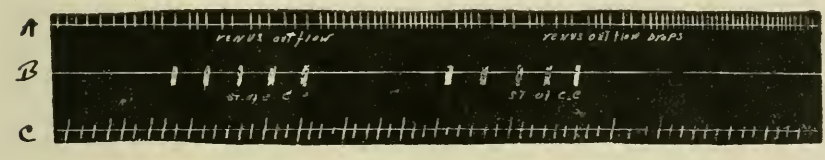

Fig. 2. Retardation and subsequent acceleration of the venous blood flow from the thyroid vein on momentary stimulation of the cervical sympathetic nerve. *A*, record of drops of venous flow. *B*, stimulation of cervical sympathetic nerve. *C*, time 3½ seconds.

outflow and by the plethysmograph. The cervical sympathetic was ligated and stimulated centrally with the same strength of current as in our stimulation experiments with the iodine determination. The results with the first method are summarized in Table IV (see also fig. 2). Even a stimulation lasting only 1–2 seconds cause a distict diminution in the venous outflow (exp. 8, 9). In some cases the vaso-constriction during the stimulation is followed by vaso-dilation on cessation of stimulation (fig. 2). The plethysmograph shows marked decrease in the volume of the gland on stimulation of the cervical sympathetic or the thyroid nerves (fig. 1) . Our experiments then confirm the work

of Ossakin (3). The cervical sympathetic supplies vaso-constrictor fibers to the thyroid.

A series of ten experiments were run in which the temporary and periodic decrease in the blood flow through the thyroid gland was produced mechanically and without any stimulation of the thyroid nerves as previously described. The iodine and total solid determination of the thyroids in this series are given in Table V. In every case there is a diminution in the percent of iodine in the lobe in which the blood flow had been periodically decreased. The decrease in iodine is not as great as in the series of stimulation of the thyroid nerves (Tables II and III). Control experiments showed that by one method of periodic compression of the superior thyroid artery there was less diminution of the blood flow through the gland than on stimulation of the thyroid or the cervical sympathetic nerves. In 80 per cent of the cases in this series the per cent of total solids is greater in case of the lobe whose blood flow was periodically diminished. It is clear that temporary vaso-constriction of the thyroid vessels, or temporary decrease in the blood flow through the thyroids induced by momentary compression of the thyroid arteries and without stimulation of the thyroid nerves produce the same changes in the thyroid gland as does the stimulation of the thyroid nerves, that is, a decrease in the iodine and water content. Since the variations in the blood flow is by itself capable of inducing these changes, and since these vascular changes are produced in the gland on the stimulation of the thyroid nerves, it is obvious that these effects of the nerve stimulation do not prove the presence of true secretory nerve fibers to the thyroid glands.

Dr. Bensley's special stain showed no difference between the control and stimulated lobes of the same gland.

TABLE I

Control series. (*Dog*)

Iodine content of right and left thyroid lobe of same animal

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH GLAND	% OF TOTAL SOLIDS	% OF I IN FRESH TISSUE	% OF I IN DRIED TISSUE	AV. DIFF. IN % OF I IN 2 LOBES FRESH TISSUE	AV. DIFF. IN % OF I IN 2 LOBES DRY TISSUE
I	R	38.2	20.5	0.03432	0.16335	0.00346	0.01686
	L	36.5	20.8	0.03778	0.18021		
II	R	26.0	19.5	0.01737	0.08914	0.00153	0.00850
	L	19.1	18.5	0.01584	0.08564		
III	R	14.5	24.2	0.30336	0.13787	0.00212	0.00982
	L	15.1	24.4	0.31024	0.12805		
IV	R	10.7	27.6	0.08886	0.32197	0.00398	0.06787
	L	10.1	33.8	0.08588	0.25410		
V	R	10.5	30.6	0.04172	0.13625	0.00172	0.02767
	L	6.6	26.5	0.04344	0.16392		
VI	R	19.2	25.0	0.00636	0.02548	0.00031	0.00078
	L	18.9	27.2	0.00667	0.02470		
VII	R	23.0	20.4	0.00732	0.03593	0.00019	0.00352
	L	18.6	22.0	0.00714	0.03341		
VIII	R	9.2	20.4	0.00666	0.03265	0.00108	0.00605
	L	10.2	20.4	0.00774	0.03870		
IX	R	53.6	20.3	0.00762	0.03754	0.00016	0.00085
	L	41.8	20.3	0.00778	0.03839		
X	R	49.7	22.0	0.01284	0.05838	0.00111	0.00744
	L	60.0	21.2	0.01395	0.06582		
					Average differences:	0.00157	0.01488

TABLE II

(*Dog*)

Effect on iodine content of thyroid of stimulation of the cervical sympathetic nerve

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	(A) % OF I IN FRESH TISSUE	(A) % OF I IN DRIED TISSUE	LOSS IN % FRESH TISSUE	LOSS IN % DRIED TISSUE	STIMULATION TIME
I	L. stim.	14.4	29.2	0.12776	0.43715	0.01499	0.04640	3 hrs. 45 min.
	R. cont.	14.2	29.5	0.14265	0.48355			Int. 10 sec.
II	L. stim.	9.4	23.8	0.03609	0.15167	0.00717	0.05800	3 hrs.
	R. cont.	8.3	21.0	0.04326	0.20967			Int. 10 sec.
III	R. stim.	13.2	25.4	0.06072	0.23905	0.01364	0.10603	4 hrs.
	L. cont.	14.2	23.0	0.07436	0.34508			Int. 10 sec.
IV	R. stim.	12.5	23.1	0.04034	0.17469	0.01179	0.07356	3 hrs.
	L. cont.	13.9	21.0	0.05213	0.24825			Int. 10 sec.
V	R. stim.	19.6	30.1	0.05498	0.18267	0.00502	0.04037	2 hrs.
	L. cont.	20.4	26.9	0.06000	0.22304			Int. 3½ sec.
VI	R. stim.	15.7	22.3	0.01664	0.07462	0.00225	0.01895	3 hrs., 30min.
	L. cont.	16.5	20.2	0.01889	0.09357			Int. 8 sec.
VII	L. stim.	31.0	23.2	0.03175	0.13680	0.00612	0.03459	3 hrs.
	R. cont.	31.4	22.1	0.03787	0.17139			Int. 10 sec.
VIII	R. stim.	38.3	28.7	0.03746	0.13052	0.00461	0.00607	3 hrs., 15 min.
	L. cont.	31.8	30.8	0.04207	0.13659			Int. 10 sec.
IX	L. stim.	10.8	24.3	0.02693	0.11087	0.00418	0.04153	3 hrs.
	R. cont.	8.1	23.7	0.03111	0.15240			Int. 10 sec.
X	L. stim.	11.9	24.4	0.01667	0.06861	0.00966	0.03974	3 hrs., 45 min.
	R. cont.	11.7	24.3	0.02633	0.10835			Int. 10 sec.
XI	L. stim.	18.1	21.0	0.01452	0.06914	0.00167	0.00795	2 hrs., 30 min.
	R. cont.	19.2	21.0	0.01285	0.06119			Int. 10 sec.
XII	R. stim.	21.0	20.9	0.00103	0.00500	0.00035	0.00166	1 hr., 30 min.
	L. cont.	22.5	20.7	0.00138	0.00666			Int. 10 sec.
			Average loss in per cent			0.00794	0.04572	

(A) Average of duplicate analyses.

TABLE III

(*Dog*)

Effect on iodine content of thyroid of stimulation of the thyroid nerves near the entrance into the gland.

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	% OF IODINE FRESH TISSUE	% OF IODINE DRIED TISSUE	% OF LOSS OF IODINE FRESH TISSUE	% OF LOSS OF IODINE IN DRIED TISSUE	TIME OF STIMULATION
I	R. stim.	12.6	23.8	0.01299	0.05450	0.00152	0.07870	3 hrs., 45 min.
	L. cont.	8.3	18.4	0.02451	0.13320			Int. 10 sec.
II	R. stim.	15.1	24.3	0.01491	0.06135	0.00238	0.01260	3 hrs.
	L. cont.	14.3	25.7	0.01253	0.04875			Int. 10 sec.
III	R. stim.	5.6	27.0	0.03883	0.14380	0.00463	0.02866	3 hrs.
	L. cont.	6.8	25.2	0.04346	0.17246			Int. 10 sec.
IV	L. stim.	3.9	22.8	0.03480	0.15263	0.00639	0.05229	3 hrs., 15 min.
	R. cont.	3.9	20.1	0.04119	0.20492			Int. 10 sec.
V	R. stim.	15.4	22.5	0.00343	0.01524	0.00103	0.00589	2 hrs., 45 min.
	L. cont.	16.8	21.1	0.00446	0.02113			Int. 3½ sec.
VI	L. stim.	12.7	21.8	0.00223	0.01022	0.00193	0.01027	3 hrs.
	R. cont.	12.7	20.3	0.00416	0.02049			Int. 10 sec.
Average loss except No. II						0.00510	0.03516	

TABLE IV

The influence of stimulation of the cervical sympathetic and thyroid nerves on the blood flow in the thyroid gland. In Exp. 1–3 the figures are given in cc. of blood per minute; in Exp. 4–9 the figures are given in number of drops of blood per 20 seconds. In Exp. 8 and 9 the stimulation lasted only 2 seconds.

EXPERIMENT NO.	BEFORE STIMULATION	DURING STIMULATION	AFTER STIMULATION
1	17.5	13	16
2	15.0	12	13
3	9.0	6	8
4	38.	28	36
5	36.	25	33
6	88	22	28
7	38	23	31
8	40	28	32 (2)
9	24	16 (1)	24 (2)

(1) 5 seconds after stimulation (2) 10 seconds after stimulation

TABLE V

The effect on the iodine content of the thyroid gland of periodic diminution of the blood flow through the gland, occlusion of the main thyroid artery for 1–2 seconds every 10 seconds.

NO. OF ANIMAL	LOBE OF GLAND	WT. OF FRESH LOBE	% OF TOTAL SOLIDS	% OF I IN FRESH TISSUE	% OF I IN DRIED TISSUE	DIFF. IN % OF LOSS IN FRESH TISSUE	DIFF. IN % OF LOSS IN DRIED TISSUE	TIME OF INTERF. CIRC.
I	Right	38.5	24.4	0.00335	0.01372	0.00272	0.01094	3 hrs., 15 min.
	L. cont.	37.9	24.2	0.00607	0.02466			
II	Left	40.2	21.1	0.00166	0.00786	0.00126	0.00719	3 hrs.
	R. cont.	28.9	19.4	0.00292	0.01505			
III	Left	3.9	20.8	0.01324	0.06365	0.00229	0.00694	3 hrs., 30 min.
	R. cont.	3.0	22.0	0.01553	0.07059			
IV	Left	14.0	25.1	0.00960	0.03824	0.00220	0.00896	3 hrs.
	R. cont.	9.5	25.0	0.01180	0.04720			
V	Right	6.5	21.6	0.00432	0.02000	0.00148	0.00723	3 hrs.
	L. cont.	11.7	21.3	0.00580	0.02723			
VI	Left	8.3	23.1	0.04281	0.18530	0.00532	0.04389	3 hrs.
	R. cont	8.0	21.0	0.04813	0.22919			
VII	Left	10.8	22.3	0.03256	0.15964	0.00422	0.02243	3 hrs.
	R. cont.	10.2	20.2	0.03678	0.18207			
VIII	Right	14.5	24.3	0.02613	0.10753	0.00671	0.03061	3 hrs., 15 min.
	L. cont.	15.8	23.7	0.03284	0.13814			
IX	Right	32.0	21.0	0.05548	0.26419	0.00111	0.00528	3 hrs.
	L. cont.	29.2	21.0	0.05659	0.26947			
X	Right	3.9	22.0	0.01280	0.05818	0.00290	0.01587	2 hrs., 40 min.
	L. cont.	3.4	21.2	0.01570	0.07405			
				Average		0.00312	0.01593	

SUMMARY

1. The iodine percentage of the thyroid lobes in dogs is practically identical (Table I).

2. Stimulation of the cervical sympathetic nerve or the thyroid nerves induces a decrease in iodine and water content of the thyroid glands (Tables II and III).

3. The stimulation of the cervical sympathetic or the thyroid nerves induces vaso-constriction in the thyroid gland (Table IV).

4. Decrease in the thyroid blood flow similar to that induced by stimulation of the above nerves, but produced by mechanical means, causes a decrease in iodine and water content of the thyroid gland (Table V).

5. The changes in the thyroids so far shown to be caused by the stimulation of the cervical sympathetic or the thyroid nerves can all be accounted for by the vascular changes.

6. No histological changes could be demonstrated following stimulation of the thyroid nerves or mechanical interference with the blood flow.

I wish to express my gratefulness to Dr. Carlson for his supervision; to Dr. Koch and Mr. Maurer for their valuable suggestions in the analytical work; and to Dr. Bensley for his help in the histological work.

ADDENDUM

These observations appear to place the thyroid gland in the same category as the liver, that is to say, as organs very delicately balanced in relation to the local circulation, irrespective of any secretory nerves. Since the periodic stimulation of the thyroid nerves induce primary vaso-constriction followed by vaso-dilatation, one cannot say at present whether the vaso-constriction or the vaso-dilation is the active factor in bringing about the discharge of iodine from the thyroid gland, or at any rate upsetting the balance between iodine absorption and iodine discharge. The thyroids have a greater blood supply than any other organ in the body. This tremendous blood supply has in all probability a far greater significance than the mere oxygen supply and the carbon dioxide removal.

The fact that relatively transient vaso-motor disturbance in the thyroid induces such marked change in thyroid activity is probably of significance in certain types of goitre in man.

The observations also offered another illustration of the difficulty in deciding whether we are dealing with vaso-motor nerves or with secretory nerves in certain organs. The adrenal glands probably also fall in this group, although most investigators interpret the increased discharge of adrenalin following stimulation of the adrenal nerves as a true secretory nerve action.

A. J. C.

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PHOTOELECTRIC CURRENTS IN THE EYE OF THE FISH[1]

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CONTENTS

I. INTRODUCTION

It is a fact well known that electrical phenomena are intimately associated with the normal activity of muscle and nerve. Of later experimental acquisition however is the knowledge that the sense organs fall within the same category; both the eye and the ear are accompanied in their normal functioning by electric currents. A beam

[1] The substance of the present paper was sent to the Arch. f. Anat. u. Physiol., July 15, 1914, under the title "Die photoelectrischen Vorgänge im Auge des Fisches," together with the original photographic records. Since the war has indefinitely delayed the appearance of the paper in that publication, no word having been received regarding it after a year has elapsed from the date of sending, I have deemed it only fair and proper that the results should be published in one of the American journals.

of light falling upon the eye sets up a complex change of electrical potential in the retina, while sound vibrations generate electric currents in the cochlea.

The frog and higher vertebrates have been the animals upon which the photoelectric currents have been most successfully studied. The phenomena which investigators have collectively brought to light by such a study are these:

When a dark-adapted eye is connected with an instantaneously reacting galvanometer, after placing one electrode on the cornea and the other on the back of the eyeball, and a brief exposure is made of the eye to light, the galvanometric deflections are, three (a negative followed by two positive) for the "make" of light, and one (positive) for the "break of light." The usage of the terms positive and negative will presently be explained. All four of these deflections are obtainable for the eye of the frog, turtle, chicken, owl, dove and monkey. Variations occur for eyes of the cat and the dog. The studies of the phenomenon in the eye of the fish have, for reasons to be mentioned, been attended with meagre success.

The galvanometers at first employed were too sluggish to respond at all to the fleeting retinal currents; and even when instruments were found which registered the electric effects for the eye of the frog, the same instruments and methods when applied to the fish were without result. Whereas the extirpated eye of the frog is capable of yielding strong retinal currents long after the operation, the extirpated eye of the fish, on the contrary, loses that ability so rapidly that, by the time the operator is ready to experiment, the currents are too feeble to record. In order to overcome this obstacle one must work with the living fish. The difficulty, however, of keeping the animal's eye slightly above water in order to apply electrodes to it, and the rest of its body below the surface in order that it may breathe, has been perhaps the chief factor which has deflected investigation of the phenomenon in the retina of the fish to a more intensive study of it in the frog and higher vertebrates. It was with the purpose of supplying this lack of data for a purely aquatic animal that the present research was undertaken.

The work was carried on in the Physiological Institute of Berlin University during the winter semester of 1912–1913. To Prof. Max Rubner, director of the Institute, I am greatly indebted for the unstinted privileges of research granted to me. The problem was proposed to me by Professor Piper, and to him for his kind supervision and valuable suggestions throughout the work I desire here to express my

thanks. I wish also to thank Dr. Arnt Kohlrausch for much kind assistance. The opportunity for the sojourn in Berlin was afforded by the grant of a Sheldon Fellowship from Harvard University.

There are certain matters of definition which should be made clear at the outset. Waller (22) found difficulty in reconciling statements of previous observers with reference to the direction of the current at "make" and "break" of light, "because that that direction was so frequently characterized as positive and negative with reference to a 'current of rest' which was itself of variable direction." Since later investigators, in agreement with Waller, have found the current of rest (i.e., the current occasioned simply by placing the cornea in circuit with the back of the eyeball) taking the direction from fundus to cornea within the eye and from cornea to back of eyeball in the outside circuit, this direction has been regarded as positive. Since in the fish eye the current of rest takes the same direction, the term positive will here be used in the same sense. In the photographic curves it is always an upward deflection.

Gotch (9) introduced the terms "On effect" and "Off effect" to designate the initial and terminal effects at make and break of light; but since his "On effect" was primarily attached to the first positive deflection in the photographic curve, I wish to employ the term to cover the negative preliminary depression and the secondary positive rise in the curve as well, unless otherwise qualified. The term "Off effect" will always refer to the simple positive deflection occurring at the break of light. In order to refer to the individual features of the curve more easily, Einthoven and Jolly (8) have been followed in denominating the negative preliminary deflection as A, the first positive elevation as B, and the secondary on-effect as C; but instead of adopting A′ to designate the off-effect (as E and J do) which associates it upon hypothetical grounds with the on-effect A, the non-partisan letter D has been given it.

II. HISTORICAL REVIEW

In the year 1849 du Bois Reymond (7) made the discovery that the retina of the fish reacted electromotorically. An extirpated eye of the tench, with one electrode on the cornea and the other either on the cross-section or on the longitudinal axis of the optic nerve, gave an electric current. An action-current could similarly be obtained by connecting the cross-section of the optic nerve with its long axis. It occurred to him to test further for the effect of light striking the retina,

but his experiment on a turtle with the aid of a multiplicator, an instrument responsive only to the stronger currents generated during muscular contractions, yielded no results.

It remained therefore for the Swede Holmgren (12, 13) to discover the photoelectric reaction for which du Bois Reymond had sought in vain. The electric currents evoked by light which he detected in the eye of the frog and the rabbit, however, he was unable to elicit with surety from the eye of the fish. The experiments were performed upon extirpated eyes of pike, perch and bleak, but the results obtained were so fleeting and irregular that, although on- and off-effects were obtained once or twice, the question remained a matter of uncertainty.

The investigations of Dewar and M'Kendrick (6) on the eyes of the goldfish, rockfish and stickleback with the aid of a Thompson galvanometer met with better success. From the goldfish (Cyprinus auratus) a positive on-effect was obtained and a negative off-effect; but whether the former was the B or C deflection was not distinguished. Similar though slower reactions were obtained for the rockfish (Motella vulgaris). In the case of the stickleback, the authors remark (p. 61): "A deflection of 400° was obtained, which was remarkably sensitive to light, the variations occurring in very short periods of time." The conclusion drawn from the experiment was that the differences in the reaction-times of the various fish was to be attributed to the different habits of the animals; or, in the authors' words, "on comparing these results, it will be seen that in the active alert stickleback, the variations occurred rapidly; in the more sedate goldfish, they occurred more slowly, while, in the case of the sluggish rockling, which hides beneath stones near low-water mark, the variations occurred very slowly."

Kühne and Steiner (16), in working with enucleated eyeballs of perch, pike, barbel and eel, obtained the positive on-effect as had already been observed by Dewar and M'Kendrick, but instead of a negative deflection at break of light, they got a feeble positive effect. When cornea, iris and lens were cut away, the posterior half of the eyeball yielded the same result, only that in this case the off-effect was decidedly positive. From the isolated retina too, provided that it was strictly fresh, were obtained similar though feebler results. In all cases the current remained constant during illumination as soon as the first positive deflection had subsided. Again the negative preliminary (A deflection) escaped notice because the instrument used was too slow in reaction to register it. It is also likely that the positive on-effect observed by these investigators was the secondary C crest rather than the more

rapid B deflection, judging from the statement, "Die Schwankungen der Retinaströme sind beim Kommen des Lichtes positiv sehr langsam wachsend, gegen das Ende rasch zum Maximum gehend, während des Belichtens anhaltend."

From 1881 to 1907 attention was concentrated upon the phenomenon as exhibited by the eyes of the frog and higher vertebrates, while study of them on the fish was neglected. The researches of v. Brücke and Garten (2), carried on with a more efficient instrument in the small Einthoven string galvonometer, were extended to cover aquatic as well as terrestrial vertebrates. The fish used were the pike and the bleak. In experiments with the former, where the electrodes were applied to the eye *in situ*, the on-effect obtained was described as "eine positive Schwankung des normal gerichtetet Bestandstromes, die während der Belichtungsdauer an Stärke zunahm," and the off-effect as "eine positive Schwankung, nach der der Strom allmählich zu seinem Ruhewert zurück kehrte." From the enucleated eyeball, however, the on-effect was negative while the off-effect was positive. For the bleak only extirpated eyes were used. The current of rest obtained was strong and ran in the outer circuit at first from the back of the eyeball to the cornea, then, diminishing rapidly to zero, flowed in the reverse or normal direction. These investigators were able to detect a negative preliminary as a feature of the on-effect, the latent period of which was 0.074 sec. in one instance, and 0.075 sec. in another. Following this negative preliminary came a positive deflection with a latent period measuring 0.128 sec. and 0.132 sec. in the two cases respectively. On darkening, the eye of the bleak yielded no definite reaction and the curve subsided gradually to zero. The combined results from both kinds of fish exhibited therefore the full set of phenomena known to occur in the frog retina, viz., the initial negative depression and the two positive deflections as the on-effect, and the positive deflection as the off-effect.

Since the work of v. Brücke and Garten no further attempt had been made to verify or add to these facts regarding the photoelectric phenomenon in the eye of the fish. The substitution of the quick-reacting string galvanometer for the older and more sluggish instruments, enabled Einthoven and Jolly (8) to analyse the currents in the eye of the frog far more carefully than had been done before, and Piper (20) to make a thorough comparative study of the photoelectric effects in the eyes of the turtle, dove, chicken, buzzard, owl, rabbit, cat, dog, monkey and octopus as well as the frog. Since the fish was not included

in this set of investigations, it was with the object of supplementing the series in that direction that, first with the aid of a d'Arsonal galvanometer and finally with a large Einthoven string galvanometer I carried out the following experiments.

III. EXPERIMENTS AND OBSERVATIONS

A. With d'Arsonval galvanometer

The kinds of fish which were used in the experiments were the tench (Tinca tinca L.) and the pike (Esox lucius L.) These are freshwater forms common to northern Germany and were obtainable throughout the winter months of 1912–13 during which the investigation was carried on. The pike, although a less hardy fish than the tench, yet, because of its larger eyes and more convenient shape, proved the more satisfactory; it alone therefore served as material throughout the chief part of the work. The fish were obtained fresh from the market every two or three days as needed and were kept in a laboratory aquarium supplied with running water.

The d'Arsonval galvanometer was employed in the earlier experiments, but as soon as the technic of working with the live animals was mastered, resort was made to the Einthoven string galvanometer in conjunction with the Hut photographic register for making the final records.

The experiments with the d'Arsonval instrument were performed on two tench and four pike. In order that the electrodes might be placed upon the eye in a dry condition, the fish was wrapped first in a wet cloth and then in a wire net to prevent any bodily movement. Thus encaged the animal was laid on its side upon bags of sand in a trough of water so as to immerse the snout and as much of the rest of the body as possible, one eye being kept above the surface. While in this position the fish could without difficulty take in water through the mouth and irrigate the gills, and it seemed to suffer no inconvenience during the hour or two of experimentation. The water was aerated with compressed air. The trough was placed in a large black box with black curtains in front. Light from a 32 C. P. electric light, set at about a meter distant, passed through an aperture in the rear of the box and was reflected from a mirror situated inside onto the eye of the fish. Before being placed in position in this dark chamber the eyeball was partially laid bare by a slight operation so that one elec-

trode might be brought in contact with the eyeball and the other with the cornea. The meshes of the wire net enveloping the fish were widened over the eye sufficiently to admit the electrodes, contact from which being made by means of short woolen threads saturated with physiological salt solution. The temperature of the water did not vary more than a degree or two centigrade from that of the room (av. 17°C.). For the first five experiments the animal was not dark-adapted; but thereafter it was placed, after the operation on the eye had been performed, in a light-proof tank of running tap-water for about three hours previous to the experiment.

TABLE 1.

Photoelectric currents in the eyes of the tench (T) and pike (P) as measured by a d'Arsonval galvanometer.

EXPERIMENT	DATE 1912	FISH	NO. HOURS DARK-ADAPTATION	DEFLECTION ON GALVANOMETER		
				Current of rest	On effect	Off effect
1	XI. 12	T. 3	0	+6. cm.	+0.5 cm.	0 cm.
2	XI. 12	T. 3	0	+4.	+2.	0
3	XI. 15	T. 3	0	+12.	+0.2	0
4	XI. 15	T. 4	0	− 3.	+ 1.	0
5	XI. 17	P. 1	0	+20.	+0.5	+0.1
6	XI. 17	P. 1	3	+25.	+11.	0
7	XI. 19	P. 1	?	−25.	+0.2	0
8	XI. 22	P. 2	3	−4. 8	−0.8	0
9	XI. 25	T. 4	1.5	+15.	+2.	0
10	XI. 26	P. 3	3	+11.	+ 6.	0
11	XII. 3	P. 4	3	?	+ several cm.	?

The results of observations made with the aid of the d'Arsonval galvanometer are given in Table I. Column three indicates the kind of fish used (T. for tench, P. for pike) and the animal's number; column four the period of adaptation to dark; columns five, six and seven give the throw in centimeters on the galvanometric scale for the current of rest[2] and for the on- and off-effects of light respectively. The plus sign signifies a current flowing in the outer circuit from cornea to back of eyeball, and the negative sign the reverse direction. It will be seen that in seven of the eleven cases the current of rest was posi-

[2] Before the beginning of each exposure this current of rest was neutralized by means of an oppositely directed galvanic current.

tive. The last, though unrecorded as to direction, was probably positive too, making a total of eight. Only once (Experiment 8) did the flashing on of the light generate a current whose direction was opposed to that of the normal current of rest, i.e., from back of eyeball to cornea in the outside circuit. In this instance both current of rest and photoelectric current were negative, accounted for no doubt by the feeble condition of the animal, for it died before the end of the experiment.

The results in general showed that the eyes of both tench and pike responded with postive currents to the onset of light and that this effect was enhanced by dark adaptation. A negative preliminary to the positive on-effect, could not be ascertained with this instrument. As for an off-effect, only once or twice could a feeble positive current be observed.

B. With Einthoven string galvanometer

The study of the photoelectric phenomena with the aid of the Einthoven string galvanometer was conducted along two lines: first, the simple effects of making and breaking the light were investigated, and then the compound effect obtained by intermittent stimulation. In the first part of the work both dark-adapted and light-adapted animals were used, but for the experiments with intermittent light only dark-adapted ones were employed. Care was taken to select from the market fish whose physiological condition was sound. This was especially important because the pike, the only fish used in this set of experiments, is not a very hardy animal, and unless procured when in prime condition yields electric currents too feeble to be photographed satisfactorily.

The procedure was modified in some respects from that which was followed in the first series of experiments. The operation was the same: a small part of the orbit overshelving the eye was cut away and the exposed muscles freed from their insertions on the eyeball so that one electrode could be brought into contact with the equatorial region. In some cases the operation was performed in the late afternoon and the fish kept in the dark over night.

An arrangement for holding the fish in an immovable position was devised as follows (fig. 1): a board of sufficient length and width to accommodate the largest animal was provided with a large hole near the end, while many smaller holes were scattered irregularly over its length. The fish was wrapped in a wet cloth, the head and gills alone

being left exposed, and then bound on its side to the board by means of wires inserted in the small holes. The under eye and gills, falling in the open space of the large aperture, were quite free from pressure, and the gills thus had freedom of action when running tap-water was led into the mouth through a rubber hose. The fish had less chance to move in this position than when enclosed in the wire net, and consequently it became somewhat numb from the confinement; but it recovered its muscular activity as soon as it was released and returned to the aquarium. When firmly fastened to the board the fish was laid in a large tray which had an outlet tube in the bottom to drain off the water issuing from the gills. This tray was situated in the large dark-box above mentioned through whose aperture at the back the light from a single Nernst glower was reflected by a mirror onto the eye of the fish. One of the electrodes was applied indirectly to the bulb of the eye by means of a wool thread, while the well moïstened clay boot of the other impinged directly on the cornea.

In order to obtain a record on the photographic paper of the moment when the light stimulus was applied, the same apparatus was used which Piper (20) had devised for his experiments on the eyes of higher vertebrates. It consisted of a T-tube (Fig. 1, *t*) containing two mirrors located in the center of the cross-arm and at right angles to each other, so that the light entering the vertical arm was reflected, half of it through one end of the cross-arm into the photographic register (*r*), and the other half through the other end onto the eye. A photographic shutter (*s*) on the end of the vertical arm made time exposures of varying lengths possible. When the shutter was opened both the eye and the photographic paper received a portion of the light simultaneously. A time-marker (*z*), making beats at the rate of five per second, was placed in front of the photographic register so that the shadow of its needle together with the shadow of the string of the galvanometer (*g*) fell upon the sensitized paper. Every photograph thus contained a three-fold record: (1) that of the begininng, duration and end of stimulation; (2) that of the retinal currents produced; (3) a time-scale in fifths of seconds.

1. Single exposures to light

(a) *Dark-adapted Eye.* In the preliminary experiments the degree of dark adaptation of the eye seemed to affect materially the size of the deflections of the galvanometric needle. In this set of experiments therefore attention was paid at first only to the phenomena exhibited

by eyes which had been adapted to the dark for at least three hours. The fixing of the fish to the board, the regulation of the artificial respiration and the placing of the electrodes on the eye, were all done by the light of a red electric globe. Since in the first experiments the current of rest in normal animals was found to flow from cornea to eyeball in the outside circuit, and the on-effect, B, to express itself by a current flowing in the same direction, it was assumed in these experiments that when the B deflection agreed in direction with that of the current of rest,

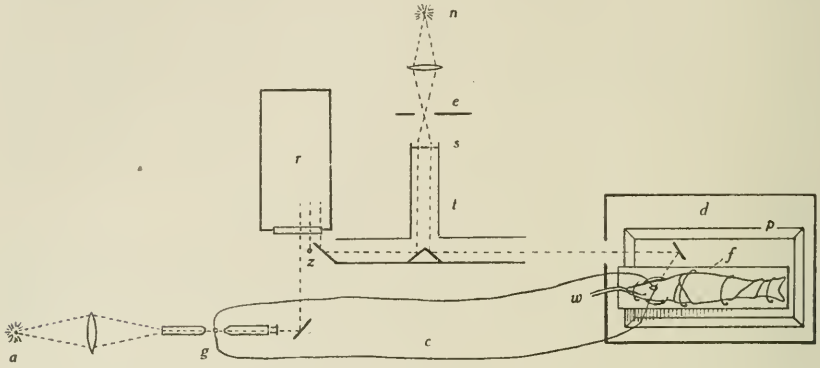

Fig. 1

EXPLANATION OF FIGURE 1

a, arc light
c, electric circuit from eye to galvanometer
d, dark box
e, episcotister
f, fish board
g, string galvanometer
n, Nernst light
p, shallow tray
r, photographic recording apparatus
s, photographic shutter
t, T-tube for dividing the light from *n*
z, time-marker

this direction was from cornea to eyeball in the outside circuit. When the direction was in disagreement, but the on- and off-effects were normal, it was assumed that a polarization current was masking the current of rest. Before the usual neutralization of the current of rest was made, the polarization current was reversed by readjusting the electrodes and brought to coincide with it. Instances of this kind however were rare. Be it understood then, that an upward deflection in the curves signifies a current flowing in the outside circuit from the cornea to the back of the eyeball.

It may be stated at the outset that the photoelectric phenomena in the eye of the fish show striking agreement with those exhibited by the frog. The on-effect comprises three features: a negative preliminary A, followed by a quick positive elevation B, and a slower secondary rise C. The off-effect consists of a positive deflection D. These phenomena will be considered first for the dark eye and then for the light eye.

The negative preliminary A appears at some stage or other in each of my eighteen series of records, although not in every individual photograph of a given series. Its absence from some of the photographs will be accounted for later. Suffice it to say at this point that it is typically present for the dark eye. It is preceded by a latent period of 0.032 seconds after the make of light, this value being an average of twenty-nine measurements obtained from the records of five different animals.

On-effect. The positive on-effect B following the negative preliminary never fails to appear except in the record of an animal whose physiological condition was at a low ebb. The latent period for B, measured from the beginning of the light stimulus to the maximum point of the negative preliminary depression, averages 0.075 seconds from thirty-five measurements. The maximum point in the deflection is arrived at on an average in 0.26 seconds. Its amplitude varied considerably in the course of the experiments owing to variations in the resistance of the electrode connections and to differences in the physiological state of the animals. The usual amplitude is about equal to that obtained from a galvanic current of one-tenth millivolt. The maximum is half again as great as this standard amplitude. In the dark-eye this positive on-effect is characteristically large. The ascending leg of the curve is always abrupt while the descending one is quite gradual. In the descending phase the curve sometimes passes below the level of the current of rest and becomes negative. The amount by which it approaches or passes under the zero line is determined largely by the strength with which the slow-developing secondary positive elevation C makes itself manifest.

In animals left in the dark over night and used on the following morning direct from their long period of dark adaptation, this secondary rise following the first positive effect is quite pronounced. With each exposure to light however, it develops at an ever retarding rate and ever diminishing amplitude. It soon disappears altogether and the whole curve following upon B subsides to a constant level which may be even

below that of the current of rest. Since in most instances the C crest developed so slowly that much photographic paper must needs have been consumed in order to obtain its full course, only a single record was made in which its full course was followed. It was a case in which the fish had been dark-adapted over night and the C effect was strong. The period from onset of light to the maximum point in the curve was here seven seconds. The descent was slower than the ascent, and after nearly twenty seconds of its downward course, at which point the record was ended, the curve had not yet returned to the zero line. The duration of exposure in this instance was two and three-fifths seconds. The condition necessary to the production of the secondary elevation seems to be a long interval of dark adaptation.

Off-effect. The positive elevation in the curve, occasioned by the shutting off of the light, has an average latent period of 0.049 seconds. The off-effect increases in magnitude the longer the eye is previously exposed to light. The ascending slope of the D crest is somewhat steeper than the descending. The latter, before reaching the level held by the curve just prior to the break of light, is often arrested in its rapid descent to continue at a more gradual rate downwards. It is quite possible that this second phase in the descent is but the underlying C elevation, upon which the off-effect had been superposed, again cropping out, or it might be due to the still lingering descent of the B crest.

(b) *Light-adapted eye.* Time did not permit an investigation of the photoelectric phenomena in the light eye to an extensive degree. The results obtained, however, are significant enough to be described. In three instances a fish was brought direct from the market, the operation performed in a large porcelain sink by the light of a 50 c.p. electric light, the fish placed in position and a photographic record made. In all three cases the first exposure revealed the fact that the off-effect was decidedly stronger than the on-effect. In one experiment an accident to the apparatus prevented the making of a photographic record, but this decided difference between the on- and off-effects was distinctly observed when the image of the string of the galvanometer was projected onto a screen. In the other two experiments reliable photographic records were secured which fully confirmed this observation. In Plate I are reproduced one set of these records.

Figures 1, 2 and 3 are curves obtained from a single animal during one experiment, showing first a typical light condition (Fig. 1), then a rapid transition to a condition of dark adaptation (Figs. 2 and 3),

in which the comparative magnitude relations of on- and off-effect have become reversed. Examined in detail the curves exhibit these features: Figure 1 (a record made immediately after bringing the fish from the market and without previous dark adaptation), the absence of both A and C deflections, the presence of a diminutive B crest and a strong D deflection; Figure 2 (a record made after ten minutes' dark adaptation following upon Fig. 1), the feeble presence of A, with B strongly developed and D much reduced—the slight elevation following B is not a C crest, as it was occasioned by the movement of the animal's body; Figure 3 (following Fig. 2 after six minutes more dark adaptation), the indisputable presence of the negative preliminary A, a further slight increase in the magnitude of B and slight decrease in the size of D. This last might be attributed to the slightly shorter period of illumination preceding it as compared with the length of exposure in Fig. 1. Thus the typical condition for the light-adapted eye is seen only in the first exposure, and it passes quickly over into that of the dark eye with a comparatively short period of dark adaptation, ten to twenty minutes.

There is another phenomenon in connection with the transition from the light to the dark condition and the reverse to which I desire to call attention. If an eye of intermediate dark adaptation be subjected to light for ten minutes, there is produced at close of exposure a gradual secondary positive rise following the D deflection which may be designated with the letter E. If during this secondary elevation an exposure of about one-eighth second be made, the A, B and D fluctuations appear in the curve without altering its general upward trend. In similar manner, if an eye of intermediate adaptation be subjected to dark for ten minutes and then the brief exposure be made, a strong downward course follows the first positive on-effect B, and upon this descending phase the D crest may be superposed as an off-effect without altering the general downward trend. The off-effect has also been obtained superposed upon the ascending phase of the C crest. Thus the D deflection may crop out upon either ascending or descending phases of other fluctuations such as the C and E crests, or upon the descending phase of the B deflection. But it is also true for the B deflection that it too may be superposed upon ascending and descending phases of C and E, and, in the case of intermittent stimulation, there are often superposed the distinct deflections of successive short-lived B-effects upon the lingering descent of the first strong B deflection (slightly exhibited in Fig. 6, Pl. II).

2. Intermittent Exposures to Light. Dark-adapted Eye

For the second set of experiments it was only necessary to interrupt the light before its entrance into the T-tube by a rotary disk of cardboard with eight radial segments cut symmetrically out of it (Fig. 1, e). In order to intercept the light simultaneously from both mirrors situated in the T-tube, the episcotister was set at the point where the light from the Nernst glower, after emerging from a condensor, came to a focus close to the mouth of the T-tube, so that each segment of the rotating disk completely and instantly intercepted the passage of the light in turn. A photographic test in which the beams of light emerging from each end of the cross-arm of the T-tube were reflected side by side onto the photographic paper, exhibited exact coincidence for the make and break of light; so that there was no uncertainty about the eye, which received the one beam, and the photographic register, which received the other, being struck simultaneously at every exposure. It was of course essential for the accurate measurement of the latent periods in the curves that this be so. The records were obtained from dark-adapted eyes exclusively in this set of experiments.

The intermittent phenomena in the eye, corresponding to the rapid interruptions of light by the episcotister, are shown in Figures 5, 6, 7 and 8, Plate II. In Figure 5, a record from animal No. 39, the rate of stimulation was three flashes per second, a frequency slow enough to allow all of the phenomena except the C variations to appear. The first D crest is either absent or masked by the first positive on-effect. The last D deflection is present although so closely superposed upon the last on-effect as to be scarcely perceptible. A slight nick, however, in the ascending slope of the last fluctuation, together with the higher altitude of its summit as compared with those preceding, is evidence of its presence. This fact is an important aid to the interpretation of the curves of higher frequency. The presence of the negative preliminary A may be inferred from the fact that the troughs preceding the on-effects are more acute than those which succeed them.

Figure 6, the fifth exposure of a series from animal No. 37, was made to intermittent light having a frequency of 16 flashes per second. The intervals of dark are slightly longer than the intervals of light, the former being 0.037 seconds and the latter 0.029 seconds on an average. Since there are 32 crests in the curve corresponding to the 32 flashes of light, they may represent either the make or break of light or else an additive effect of both. An analysis of the curve will be found on

p. 388, in section C of division V. When the frequency was increased from 16 to 28 stimulations per second, the eye ceased to respond with individual currents and the general effect produced was the same as that for continuous light, and is exhibited in Figure 7.

In order to determine whether the eye by this time might not be fatigued, the frequency was lowered to 25 flashes per second, and it was found that the responses of the eye, although feeble, were again recordable. The sensitiveness of the galvanometer during this series is shown in Figure 8, a curve produced by a constant current having a strength of one millivolt.

IV. SUMMARY OF OBSERVATIONS

1. The photoelectric phenomena in the eye of the fish (pike) are quite similar to those in the frog. The current of rest flows from cornea to back of eyeball in the outer circuit, a direction designated as positive. The onset of light evokes in the curve a negative preliminary depression A, a positive deflection B and a secondary positive effect C. The withdrawal of light occasions a single positive deflection D.

2. The initial depression A appears only after a certain amount of dark adaptation; its magnitude is relatively small, and it has a latent period of 0.032 seconds.

3. The B deflection is feeble in a light-adapted eye, but with ten to twenty minutes' adaptation to darkness it becomes five to seven times as large. Its latent period is 0.075 seconds and its maximum is reached on an average in 0.26 seconds.

4. The C deflection appears only in a thoroughly dark-adapted eye; its development is slow and becomes ever slower with every exposure to light. Its magnitude may, after long adaptation to dark, exceed that of B by two or three times. In one measured instance the summit was reached in seven seconds.

5. The off-effect D increases in size with the length of previous illumination of the eye. Its latent period is 0.049 seconds and its summit is reached in 0.165 seconds.

6. To intermittent light the eye yields separate responses up to a frequency of 25 flashes per second. At 28 per second the responses become blended together so that the curve obtained resembles that produced by exposure to continuous light, the A and B deflections of the first on-effect and the last off-effect alone being distinguishable.

7. A change in the direction of the current of rest may occur through

changes in the physiological state of the fish forerunning death, or by gradual change in the polarity of the electrodes.

V. DISCUSSION

A. Criticism of the Method

The factors which tended to vary throughout the experiments were the contact of the electrodes with the eye, the tension of the string of the galvanometer, the intensity of the light and possibly the oxygen supply. The temperature of the water conducted into the fish's mouth was that of ordinary tap water and varied from 15 to 17 degrees centigrade. Although in cases of extreme changes the temperature may decidedly alter the phenomena [Gotch (9), Nikiforowsky (18)], this fluctuation from day to day was too slight to modify the reactions in any way. The fish, bound to the board so as not to injure it by too strong compression, was capable of slight muscular movements, and these together with the respiratory action of the gills, affected the contact occasionally between the electrodes and the eye; but as muscular movements of a disturbing character were only occasioned by lack of oxygen, when the water supply was regulated properly the fish lay quiescent throughout the experiment. In order to obtain the best results the tension of the galvanometer string often had to be altered in the course of the same experiment, loosened when maximum throws were desired and tightened when the fleeting currents set up by a high frequency of stimulation demanded a quicker response. Since the light from the Nernst glower was reflected into the eye by means of a small adjustable mirror located inside the dark chamber, the intensity varied to some extent with every readjustment.

The specific effects which an increase in light intensity has upon the curve are summed up in the recent work of Brossa and Kohlrausch (1) in these words:

> Die Latenz wird kürzer, der negative Vorschlag tritt erst von einer bestimmten Lichtintensität auf und wird von da an stärker ausgeprägt. Alle drei positiven Stromschwankungen . . . . steigen rascher und steiler an; die Eintrittsschwankung und die Verdunklungsschwankung erreichen früher ihren Gipfel. Die Senkung zwischen positiver Eintrittsschwankung und sekundärer Erhebung wird tiefer. Die Verdunklungsschwankung steigt in immer schärfer werdendem Knick von der Abszisse aus an. Wohl gemerkt gehen alle diese Formveränderungen Hand in Hand mit einer Zunahme der Ordenatenhöhe der Ausschläge.

Thus an increase of light intensity produces a shortening of the latent periods, a steepening of the slopes of the deflections and an increase in their altitudes.

Although these variable factors probably affected the finer character of the curves to a certain degree as just mentioned, they did not alter their general structure nor the direction of the deflections. In the following discussion which deals with the structural features of both simple and intermittent curves rather than with their magnitude relations, the latter will for the most part be left out of consideration.

B. Comparison With Results Obtained for Other Animals

If we compare the curves obtained from the eye of the pike with those obtained from the frog, we find that for both simple and inter-

TABLE II.

A comparison of the latent periods of the photoelectric reactions for the eyes of the bleakfish, frog and pike.

INVESTIGATOR	ANIMAL	LATENT PERIOD OF A DEFLECTION	LATENT PERIOD OF B DEFLECTION	FROM MAKE OF LIGHT TO MAXIMUM OF B DEFLETION	LATENT PERIOD OF D	FROM BREAK OF LIGHT TO MAXIMUM OF D	BLENDING RATE FOR INTERMITTENT STIMULATION
v. Brücke and Garten	bleak	0.074″ 0.075	0.128″ 0.132				
Piper.....	frog	0.045	0.085	0.3″	0.05″	0.18-0.27″	15 per sec.
Day.....	pike	0.032	0.075	0.26	0.049	0.165	28 per sec.

mittent stimulation the phenomena are closely similar. The corresponding deflections are all like in sign. In Table II are given for comparison the latent periods for the A, B and D deflections, together with the time required for the B and D crests to attain the maximum points in their courses.

The first set of values is that obtained by v. Brücke and Garten (2) on the bleakfish (Bleie), the second set by Piper (20) on the frog, and the last by myself on the pike.

Since the same string galvanometer had been used by Piper as was used in the foregoing experiments, it is noteworthy to compare especially

the last two sets of values. It should be first remarked however that v. Brücke and Garten employed a smaller and considerably less sensitive string galvanometer than Piper did. Furthermore, since they succeeded in obtaining but two photographic records for the bleak, measurements of which are given in the table, their results are to be accepted with reserve.

Turning then to the second and third sets of values, it will be seen that both the latent periods and the times required for the on- and off-effects to reach their maxima, are greater in the frog than in the fish; also that the amphibian eye ceases to respond to intermittent stimuli at a lower frequency than the fish eye does.

These facts might lead one to suppose that the photoelectric processes in the visual organ of the pike were of a more rapid nature than those of the frog. Considering the facts however that the values for the fish were obtained from eyes *in situ* and still in communication with the circulatory system, while those for the frog were obtained from extirpated eyes; and that Piper employed a light of stronger intensity (five ampere arc) than I did, the values in the table are not so closely comparable. The interruption of the circulation in the eye through extirpation presumably occasioned a retardation of the photoelectric reaction, while the higher intensity probably had an accelerating effect which tended to shorten the latent periods; but there is no way of estimating how much the acceleration compensated for the retardation. As a matter of opinion, I believe that the compensation was not enough to make up for the depression in irritability induced by impairing the metabolic activities of the retina.

The value 28 in the last column, the frequency of intermittent flashes at which a blending of the individual deflections in the retinogram occurred, was not an average but was a single value obtained in the course of one experiment upon a normal vigorous animal. Experience taught that much depended upon the tension of the string in the galvanometer in securing the fleeting currents of the eye. Thus currents of high frequency to which the slack fiber would not respond, could be recorded on stretching it tighter.

Piper (20) found that dove and buzzard eyes responded to as high as 40 flashes per second, the chicken to 35, the owl to 20, the rodent to 25, the cat and dog both to 25 and the Makakus Rhesus monkey to 17. That the fish, then, a cold-blooded animal, should possess an eye which reacts with greater rapidity to photic stimuli than do those of warm blooded animals like the rabbit, cat, dog or monkey, seems

incredible, because the fact that the rate of nerve transmission in the cold blooded forms is slower, would lead one to expect a correspondingly slower reaction on the part of the retina as well. Further, the fact that the latent periods for the on- and off-effects in the eyes of the mammals mentioned are about twice as short as those for the fish, would likewise indicate that only minimal intermittent values have as yet been obtained for the warm blooded animals, because theoretically the animal with the shorter latent period is physiologically better capacitated to react to a higher rate of intermittent stimulation than an animal with a slower reaction period. Since various drugs were employed by Piper in his operative methods, such as ether for narcotizing, curare for inhibiting muscular movement, and atropin for enlarging the pupil, these may have played a rôle in diminishing the sensitiveness of the eye to light. As to the use of atropin, Gotch (9) states definitely that it lengthens the latent periods. Other factors, too, such as the tension of the galvanometer string, the resistance of the electrodes, the intensity of the light, the physiological condition of the animal, etc., enter in to make difficult a close comparison between the results obtained by Piper on birds and mammals and those here given for the fish.

C. Synthesis of the oscillatory curve

An analysis of the deflections produced in the retinogram by intermittent stimulation has been carefully made by Piper (20). He regards the curve first from the standpoint of photoelectric currents set up by a series of "flashes" of darkness, and again as compounded of the serial effects from a sequence of flashes of light. By both methods of approach he arrives at the conclusion that the curve consists chiefly of negative preliminaries plus positive off-effects, i.e., of A and D deflections alternating in rapid succession. If the rate of stimulation is slow, the B deflections predominate; but since with increase of frequency the B effects diminish in amplitude while the A and D deflections retain practically their original size, the curve comes gradually to be composed of the last two kinds of deflections. His argument is based upon three facts revealed by measurement on the intermittent curve: first, the time elapsing from each make of light to the beginning of each depression is identical with the length of the latent period for the negative preliminary, A; second, the period elapsing from break of light to the maximum point of the next succeeding depression is identical with the latent period for the positive off-effect, D; third, when an intermittent

series ends in an off-effect, the last deflection, positive, corresponds in length of latent period to that of a deflection appearing at the end of a continuous exposure, thus making the two identical. In the oscillatory curve of the frog, therefore, it is the A and D deflections which primarily determine the oscillations when the frequency of stimulation is relatively high.

From a similar analysis of my own curves I have arrived at a like conclusion in regard to the synthesis of the intermittent curve for the eye of the fish. Figure 6, Plate II, will serve to illustrate. As the duration of each flash in this curve is about the same as that of the last flash in Figure 5, Plate II, which, as has already been pointed out,[3] occasioned a superposition of the D deflection on top of a B deflection, one may infer that such is probably the case here too, every upward stroke being compounded of the ascending slopes of the B and D elevations. There is however another factor involved which tends to diminish this additive effect. It is the negative preliminary A, which exerts a depressing influence upon the positive phases of the curve in the following way: since the sum of the interval of darkness following a stimulation plus the latent period of the next ensuing A deflection (i.e., 0.029 + 0.02″) is always less than the latent period (0.09″) needed by the off-effect to arrive at a maximum, the A depression curtails the full additive value of each off-effect. Therefore the summits of all the upward deflections in the curve, as soon as the effect of the first strong positive on-effect has subsided enough to allow them to appear, are necessarily submaximal up to the last one; but this final one, because of the fact that no A deflection follows to depress it, rises to a maximum. The more the rate of stimulus is increased, the more the A deflections overlap the B deflections and tend to diminish their altitudes.

Further, since the altitudes of B and D deflections both are dependent upon the restorative periods of dark and light preceding each respectively, according as these periods are shortened the altitudes of the crests are diminished. It follows then that two factors, one of interference and the other a diminution in the length of the restorative periods after each stimulation, combine to depress the crests of the waves to a common level as the frequency of stimulation is increased. The rise at the end of an intermittent series is an expression of the release from the depressing effect of a subsequent negative preliminary.

[3] See p. 382.

D. Interpretation of the retinogram

It is generally conceded that the C deflection can play no part in producing the sensation of light, because of the slowness with which it develops. The underlying cause for this deflection might be one of several phenomena which take place in the eye: the chemical dissociation of visual purple, the migration of pigment, the contraction of cones or the chromolytic changes said to occur in the ganglion cells of the retina.

As to the first phenomenon, Kühne and Steiner (16) state that the photoretinal currents occur in the frog after the visual purple has been bleached out. This is confirmed by Holmgren (14). The former authors also claim that if during dark adaptation the re-formation of visual purple be prevented by lowering the temperature, the eyes do not then exhibit any gain in sensitiveness, but react like light-adapted eyes. From the general nature of the statements, however, it is impossible to say whether it is the B and D deflections alone which are affected, or whether C is also.

Since visual purple and the C deflection are both characteristic for dark-adapted eyes of many animals, two questions arise: first, whether visual purple is present in the eyes of all animals whose retinas, when dark-adapted, yield the C deflection upon exposure to light; and second, whether the C deflection is obtainable from all retinas which exhibit visual purple. An answer to these queries must await the accumulation of more experimental data on the subject.

In regard to whether the migration of pigment offers a basis for the explanation of this slow-developing fluctuation in the curve, the number of facts at hand from which to judge is meagre. The migration of pigment and the C deflection are both elicitible from the dark-adapted eye of the fish, frog, dove and lobster. In the retina of the dog on the contrary, neither phenomenon occurs, for, according to Chiarini (4), there is no evidence of photokinetic activity in the pigment, and upon the statement of Piper (20) the C deflection is either entirely absent or else is very small. In the pig's eye the migration of pigment is very slight according to Van Genderen Stort (21); and in the case of the cat the same thing is probably true, judging from the scant amount of pigment present in the epithelial layer. No retinogram has ever been made for the pig, but for the cat v. Brücke and Garten (2) and Piper (20) have photographically recorded pronounced C deflections. Although Hesse (11) among others has described a migration of pig-

ment in the cephalopod eye under the influence of light, the photographic curves of the photoelectric effects obtained by Piper (20) from cephalopod eyes reveal not the slightest trace of a C deflection.

Thus the evidence, although inconclusive because the two phenomena have not been studied side by side in the same animal and under similar conditions, makes it rather improbable that the photokinetic activity of the pigment underlies the phenomenon of the C deflection in the retinogram. A study of the photoelectric effects in the eye of an albino rabbit where pigment is entirely lacking, would throw direct light on the problem. For the common rabbit the C crest has already been recorded photographically by Piper (20).

The contraction of the cones of the retina, observed for the eyes of the fish, frog, salamander, lizard, dove and pig [Van Genderen Stort, (21); Chiarini (3, 4); Hertel (10)] might come into consideration in seeking an explanation for the C deflection, were it not for the fact that, although the latter phenomenon occurs in the arthropod eye [v. Brücke and Garten, (2)], no such contraction of retinular elements takes place in it under the influence of light [Parker (19); Day (5)]. In palaemonetes Parker claims that, although the "proximal pigment cells" undergo no change in length through exposure to light, the "distal pigment cells" become contracted, and the "accessory pigment cells" exhibit migratory activity. But if, as has previously been shown, the mechanical movement of pigment granules can not account for the C deflection, it is no more to be expected that any other kinetic activity, whether of contraction or the shifting of cellular elements, should account for it. It is more probably to be explained upon the basis of a chemical reaction of some substance such as visual purple, or possibly in terms of the chromolysis in the retinal ganglion cells which, according to the observations of Chiarini (3, 4), occurs under the action of light. Until the substance can be definitely identified which underlies the phenomenon of the C deflection, it goes by the general designation of the "Third Substance" in the theories of Einthoven and Jolly (8) and of Piper (20).

In regard to the other deflections in the curve it is impossible from the experimental data at hand to conclude just what their relations to the visual sensations are. Ishihara (15) has argued that they can not be correlated with sensations, first, because they are of too brief duration to represent continuous sensations, and second, because the D deflection which should represent a sensation of darkness is the same in sign as the B deflection which theoretically represents the sensation of light. But the premises upon which these conclusions are based

are debatable issues themselves. The fact that the B and D deflections are alike in sign does not necessarily exclude all correlation between them and the sensations of light and darkness respectively, if one assumes them to be produced by the reactions of two substances which, although reacting with like directional sign as to the electric currents produced, possess a qualitative difference which the galvanometer is incapable of expressing. Were the instrument, for example, to record the B deflection as a red curve and the D deflection as a blue one, the directional sign remaining the same, any such qualitative difference would immediately be apparent; thus red could be associated with the sensation of light and blue with that of darkness.

As to the first argument, the premise that sensations of light are continuous, is invalid in the sense of an unvarying continuity. It was however evidently with that sense in mind that the argument was formulated. According to Ishihara, for example, the sensation which a person experiences when an electric light is turned on is a continued, unchanging sensation of brightness which lasts until the light is turned off. An analysis of such a sensation however discloses the error of the premise. When one gazes at the sky one may think that with respect to brightness the sensation thereof suffers no apparent change; but one needs only to perform two simple experiments in order to convince himself to the contrary.

In a room having a window which commands a free expanse of sky, cover one eye, hold a card in front of the other so as to screen off half the patch of sky, gaze fixedly at a speck on the pane for a moment and then withdraw the card. The half of the sky at which you were gazing is seen to be distinctly dimmer than the one just uncovered. Again, in a very dimly illuminated room, when one gazes at a white spot on a black background, the spot soon vanishes like an extinguished light. The element of fatigue, therefore, both for the retina as a whole and for the fovea centralis, makes quite impossible a continuous sensation of light in the sense of an unchanging sensation. Under ordinary conditions the eye is ceaselessly shifting on its axis so as to bring now this now that portion of an object to fall upon the fovea for close inspection, so that the factor of fatigue is reduced to a minimum.

The sensation of darkness is likewise assumed by Ishihara to be continuous, and that on that account the D deflection can not be the correlative of the sensation. But one may gaze at a black field as well as at the sky, cutting off half of the view with a card, and find upon removing the card that the newly exposed half is comparatively the

darker, thus showing that the sensation of blackness steadily diminishes in intensity as its duration increases.

The decline of a deflection after reaching its maximum altitude might therefore be the expression of the diminution in strength of a sensation, whether of light or of darkness. The ascending phase of a deflection would analogously indicate the growth of a sensation to a maximum. In the foregoing I have had B in mind rather than A as being the representative of the sensation of light; but that the A deflection can not be ignored will be apparent from an analysis of the intermittent curve.

When one compares the threshold value for the fusion of visual images with that for the blending of the oscillations in the intermittent curve, it is found that there is a certain approximation between the two. In the former case the threshold value for the human eye ranges, according to Piper, from 20–50 stimulations per second, at which frequency the intermittent flashes give the sensation of continuous light, while in the latter case the value ranges, depending on the animal, from 17–40 flashes per second, at which the individual oscillations blend into a smooth curve. From these comparative values one may infer that some general correlation exists between the photoelectric phenomena and the visual sensations of light and darkness. They do not however throw much light on the question of the significance of the individual deflections.

If the oscillatory curve is formed synthetically as has been described, then the sensations in the brain corresponding to the oscillations must be due, those of light to the depressions and those of darkness to the crests in the curve; because, from the analysis of the curve, the A deflection plays the chief rôle in creating the depressions and the D deflections the crests. The B deflection here seems to participate but little and consequently has little to do with the sensation of light. Nikiforowsky (18) has shown furthermore that the B deflection vanishes completely when the temperature is lowered to zero degrees centigrade, while the A and D deflections remain almost unaffected. According to this again, the A and D deflections, being the most stable, would represent the sensations of light and darkness respectively; because, were the sensation of light to be attributed to the B deflection, when the latter vanished upon lowering the temperature it would be a case of *reductio ad absurdum*, of an eye capable of rendering a sensation of darkness but incapable of rendering one of light. In the simple curve for the normal eye, however, the B deflection is such a prominent feature

that further investigation is needed to decide whether it can be left entirely out of consideration or not.

The preceding experiments dealt, it must be remembered, with dark-adapted animals (with but one exception), so that both simple and oscillatory curves must yet be studied for the effects of light-adaptation before a definite theory can be well grounded.

E. Similarity of the tri-substance theories

In looking over the literature there have come to my attention certain facts in regard to the theories proposed by Einthoven and Jolly on the one hand and by Piper on the other which are worth drawing attention to. The two theories are supposed to be antagonistic to each other, but they have in fact more in common than has been suspected.

When Piper evolved his theory it was with the object (p. 120) of obviating the necessity of ascribing each fluctuation in the curve to a separate process in the retina, as he suppposed had been done by Einthoven and Jolly. I think however that a careful analysis of the two theories will show that Piper partly misinterpreted the application of the theory of the other two men, and that what he sought to obviate was something which he had imputed to their theory which did not in reality belong there.

Table III will enable us easily to compare the two substance theories. On the left side are given the characteristics of the substances as conceived by Einthoven and Jolly, and on the right as conceived by Piper. Substances II, I and III of Piper's are comparable to I, II and III of the Einthoven-Jolly theory. In each cross comparison it will be noted that the direction of the reaction is the same for the lighting effects and also for the darkening effects and further that the latencies of reaction for the several substances likewise correspond in the matter of relative duration. During illumination the curves for Piper's substances II and I, after having attained a maximum positive and negative level respectively, remain constant on that level up to the off-effect, whereas, according to Einthoven and Jolly they do not remain constant, substance I continuing in the original direction and becoming more and more negative, and substance II changing direction after reaching a maximum positive value and returning to zero. Except for this difference in the continuous effects during the exposure, the three substances of the one theory have identically the same characteristics as

the three corresponding ones of the other theory. Thus Piper's substance II is nothing more nor less than substance I of Einthoven and Jolly, and vice versa. The third substance is identical for both.

An apparently radical difference between the two theories comes to light when the theoretical reactions of the substances are applied

TABLE III.

The tri-substance theory of Piper compared with that of Einthoven and Jolly.

EINTHOVEN AND JOLLY		PIPER
Substance I		Substance II
Lighting effect	negative	negative.
During exposure	increasingly negative	constant negative level.
Darkening effect	positive	positive.
Latency of reactions	relatively short	relatively short.
Requirements	light eye exposed to a flash of darkness	
Substance II		Substance I
Lighting effect	positive to a maximum	positive to a maximum.
During exposure	diminishingly positive	constant positive level.
Darkening effect	negative	negative.
Latency of reactions	medium	medium.
Requirements	dark eye and short exposure to weak light.	
Substance III		Substance III
Lighting effect	positive to a maximum	positive to a maximum.
During exposure	diminishingly positive	diminishingly positive.
Darkening effect	none	none.
Latency of reaction	long	long.
Requirements	dark eye and relatively strong illumination.	

to the retinogram. In the theory of Einthoven and Jolly the reactions of the three substances apparently occur in sequence, while by the Piper hypothesis they progress simultaneously. It would seem, then, to be a difference between driving horses tandem and driving them abreast. It is true there is a commencement sequence for the reactions of the three Piper substances, but the intervals between the beginning-times

of each are relatively short. In the construction which Piper placed upon the statements of Einthoven and Jolly concerning their curves on the contrary, the reactions of substance I proceeds to its finish before the reaction of substance II begins. This seems at least to have been the conception of the E and J. theory held by Piper when, in referring to it (p. 120), he judged it as physiologically impossible because it demanded a separate retinal process to account for each variation in the curve. In opposition to this idea it appeared therefore more probable to Piper that the fluctuations in the curve were interference phenomena resulting principally from two almost simultaneous reactions acting in opposite directions and at slightly different rates; and upon the basis of this conception he evolved a graphic schema by which he explains the genesis of both simple and oscillatory curves.

Although this interference conception was developed and formulated into definite terms by Piper, its essence was nevertheless present in the theory of Einthoven and Jolly. That these investigators had also conceived of interference occurring between overlapping reactions will be seen from the following quotations (in which all italics are mine).

"The lighting effect (p. 400) of the first substance . . . does not yet appear in fig. 14, which need not surprise us, since the darkening reaction must appear sooner than the lighting reaction. Nevertheless the lighting effect makes itself appreciable to some extent during the record of the curve; for . . . . it is evident that the summit height in fig. 14 is only little greater than that in fig. 13. The increase is only 6 on 110 microvolts, while the duration of lighting is increased from 1.9 to 3 sec. It is the lighting effect of the first substance which here *hinders* the development of a higher summit B." Again on p. 401, "the summit A, is *superposed* in such a way upon the slow wave C that the form of the waves may easily be recognized separately." On p. 402 the interference conception is more expressly formulated in the statement, "If the duration of the lighting is a little longer and the effects of the other two substances begin to become perceptible, the off effect is determined by the *resultant of three forces*."

From these citations it is clear that the nucleus of an interference hypothesis existed in the minds of Einthoven and Jolly but that it was never developed to the extent that it was later by Piper.

In many of my records the continuous effect of illumination is not expressed in the curve as an undeviating course on a constant level, but by a steady and continuous descent, a phenomenon not interpretable upon the basis of the Piper theory that the reactions of substance I

and II remain in a state of equilibrium during the period of illumination. Either substance I must diminish in the strength of its positive reaction or else the reaction of substance II must steadily grow in strength negatively, in order to account for this fall in the curve which seems to be a constant feature in retinograms of dark-adapted fish eyes. Nor can the difference between the reactions of dark and light eyes, a phenomenon not taken into consideration by Piper in testing his theory, be otherwise accounted for than by the foregoing assumption. I am therefore inclined to agree with Einthoven and Jolly that the reactions of substances I and II, instead of reaching a level of stability, vary constantly, either waxing stronger or growing weaker, throughout the period of illumination.

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DESCRIPTION OF PLATES

The figures in the two plates are tracings from photographic records.

Plate I

Fig. 1. Experiment 40. Date, '13/III/7. 12.05 p.m. Exposure No. 2. The fish was brought fresh from the market and photographic records were made immediately. Exposure No. 1 yielded a curve similar to No. 2 but the latter was selected for reproduction because of its clearer image. This record was made after the eye had been adapted to the Nernst light for two minutes to counteract the two minute interval of dark adaptation necessitated by the development of exposure No. 1. The off-effect for the light-adapted eye is here seen to be stronger than the on-effect. B and D deflections manifest.

Fig. 2. Experiment 40. Date, '13/III/7. 12.18 p.m. Exposure No. 3, following exposure No. 2 (fig. 1) after 10 min. dark adaptation. The on-effect is now stronger than the off-effect. B and D deflections present and a faint suggestion of A.

Fig. 3. Experiment 40. Date, '13/III/7. 12.27 p.m. Exposure No. 4, following expos. No. 3 after 7 min. more dark adaptation. Deflection A is now fully present, B has increased in strength while D has grown feebler.

Fig. 4. Experiment 39. Date, '13/III/6. 11.33 a.m. Exposure No. 1. Fish dark-adapted over night. A, B, C and D deflections present. Although the D deflection in this exposure is stronger than the B, in the other exposures of this experiment the reverse relation is found. The larger D deflection may, in this case, have been due to a slight condition of light adaptation at the beginning. This light adaptation was not however strong enough to repress the C deflection.

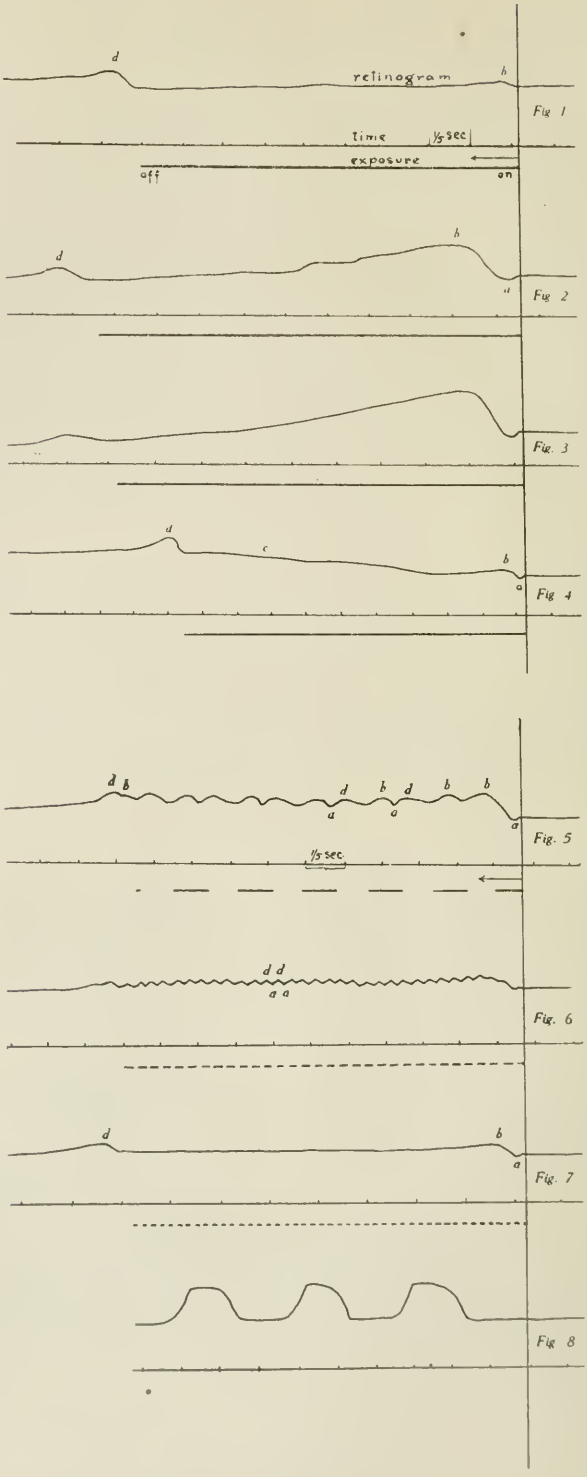
Plate II

Fig. 5. Experiment 39. Date, '13/III/6. 12.04 p.m. Exposure No. 4. Fish was kept dark-adapted after each of the previous exposures. The illumination, recorded by the dashes in the lowest line of the figure, was intermittent, the rate of stimulation being about three flashes per sec. The duration of a single flash is about 1/6 sec. A, B and D deflections are present. The last D deflection is superposed upon the last B deflection owing to the curtailment of the final period of illumination.

Fig. 6. Experiment 37. Date, '13/III/4. 4.27 p.m. Exposure No. 5. Fish was dark-adapted over night. Rate of stimulation, 16 flashes per second. Intervals of light = 0.029 sec., of darkness = 0.037 sec. To the 32 flashes of light there correspond 32 crests in the curve.

Fig. 7. Experiment 37. Date, '13/III/4. 4.47 p.m. Exposure No. 6. Rate of stimulation, 28 flashes per sec. The individual oscillations have fused and the effect is the same as for continuous illumination.

Fig. 8. Experiment 37. Date '13/III/4. 5.25 p.m. The three deflections were produced by substituting a galvanic battery for the eye of the fish and sending three consecutive shocks through the galvanometer having a current strength of 0.1 millivolt. The current from the eye throughout Experiment 37 gave throws of scarcely half this altitude.



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THE COMPARATIVE RATE AT WHICH FLUORESCENT AND NON-FLUORESCENT BACTERIA ARE KILLED BY EXPOSURE TO ULTRA-VIOLET

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The source of ultra-violet used was a Cooper-Hewitt quartz mercury-vapor burner operating at 170 volts, 3.3 amperes and 2400 candle-power. The non-fluorescent bacteria used were B Colil communis, B. violaceous, B. Proteus vulgaris, B. sulbtis, Sarcina aurantiaca, Micrococcus capsulatus, and B. mucosus capsulatus. The fluorescent bacteria were furnished by the State Water Survey of Illinois. The Water Survey obtained the bacteria from the samples of water sent in from different parts of the state for examination. Mr. Tanner of the Water Survey had not gone far enough in the classification of these bacteria at the time we obtained them from him to furnish the names for them and for that reason they were referred to in this paper as fluorescent bacteria numbers 1, 2, 3, 4, 5, 6 and 7. He had determined, however, that they were all different types of fluorescent bacteria and were non-spore formers.

The liquids containing the bacteria to be exposed to ultra-violet were prepared in the following way. A standard loop of bacteria was taken from a five day old culture and introduced into 25 cc. of 0.8 per cent sterile sodium chloride solution. This liquid was filtered through a fine grained sterile filter. Two cc. of the clear filtrate containing the bacteria were introduced into each of eleven quartz tubes. These tubes were placed 30 cm. from a Cooper-Hewitt quartz mercury-vapor burner operating at 2400 candle power. At intervals of 20 seconds a tube was removed. In this manner tubes containing the bacteria were obtained which had been exposed to the light for 0, 20, 40, 80, 100, 120, 140, 160, 180, 200 seconds respectively. The contents of these tubes were plated with agar and incubated at 37° C. for 48 hours. At the end of this time counts were made according to the ordinary bacteriological method.

Results of these counts may be seen in Table 1 for eight kinds of fluorescent bacteria and in Table 2 for seven kinds of non-fluorescent bacteria. The numbers of colonies for the 20 and 40 second intervals of exposure are not given, because as a rule they were too numerous to be counted accurately. The counts for the numbers of colonies after 0 time of exposure were made possible by diluting the liquid a million times before plating.

It may be seen in Table 1 that there were alive 31 colonies of fluorescent bacteria No. 1, 15 of No. 2, 3 of No. 3, 3 of No. 4, 11 of No. 5, 2

Table I. Fluorescent Bacteria

Time of exposure	Number of bacteria No.1	Number of bacteria No.2	Number of bacteria No.3	Number of bacteria No.4	Number of bacteria No.5	Number of bacteria No.6	Number of bacteria No.7	Number of bacillus pyocyaneus
0 Sec.	72,000,000	80,000,000	28,000,000	70,000,000	75,000,000	33,000,000	25,000,000	79,000,000
20 "	----------	----------	----------	----------	----------	----------	----------	----------
40 "	----------	----------	----------	----------	----------	----------	----------	----------
60 "	750	813	55	210	460	12	361	68
80 "	459	623	29	79	211	10	112	20
100 "	227	221	25	56	113	7	18	14
120 "	128	102	20	29	103	5	8	12
140 "	97	88	15	18	56	4	6	8
160 "	81	73	12	13	38	3	6	2
180 "	43	29	10	6	25	3	5	1
200 "	31	15	3	3	11	2	4	1

Table II. Non-fluorescent Bacteria

Time of exposure	Number of B.Subtilis	Number of B.Mucosus capsulatus	Number of B.Proteus vulgaris	Number of B.Violaceus	Number of Micrococcus capsulatus	Number of B.Coli communis	Number of Sarcina aurantiaca
0 Sec.	13,000,000	24,000,000	295,000,000	27,000,000	28,000,000	26,000,000	195,000,000
20 "	----------	----------	----------	----------	----------	----------	----------
40 "	----------	----------	----------	----------	----------	----------	----------
60 "	13	460	2,480	83	171	378	4,820
80 "	7	112	79	60	4	9	60
100 "	7	107	71	20	2	1	10
120 "	6	8	4	5	2	0	2
140 "	4	6	2	4	1	0	1
160 "	2	5	2	0	0	0	1
180 "	1	0	1	0	0	0	0
200 "	0	0	0	0	0	0	0

of No. 6, 4 of No. 7, and 1 of B. pyocyaneus after an exposure of 200 seconds to ultra-violet while in Table 2 it may be seen that all of the non-fluorescent bacteria were killed when exposed for a similar length of time. This is taken to mean that the fluorescent bacteria are more resistant to the effect of ultra-violet than non-fluorescent bacteria.

The results of these experiments are also expressed in the form of curves in figures 1, 2, 3, 4, 5, 6, and 7. The unit of the ordinate scale is 1 cm. to one hundred bacteria, that of the abscissa is 1 cm. to 20

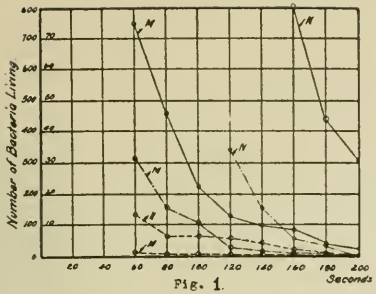

Fig. 1.

——— Fluorescent bacteria No. 1

– – – – Non-fluorescent B.subtilis (two days old)

– – – Non-fluorescent B.subtilis (two weeks old)

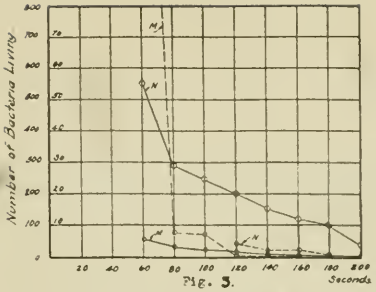

Fig. 3.

——— Fluorescent bacteria No. 3

– – – – B.proteus vulgaris

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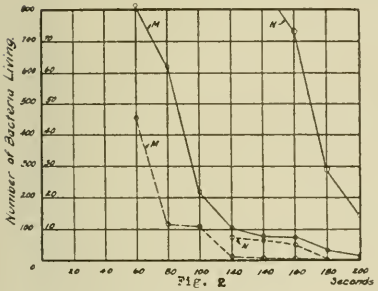

Fig. 2

——— Fluorescent bacteria No. 2

– – – – B.mucosus capsulatus

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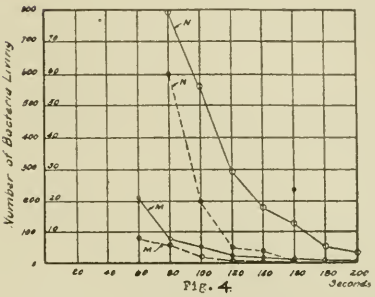

Fig. 4.

——— Fluorescent bacteria No. 4

– – – – B.violaceus

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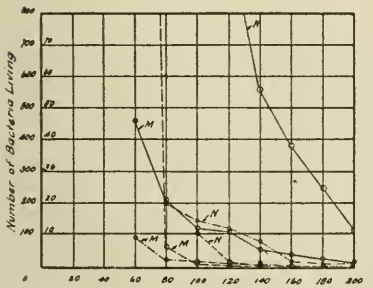

Fig. 5

——— Fluorescent bacteria No. 5

– – – – Sarcina aurantiaca

—·—·— B.pyocyeneus

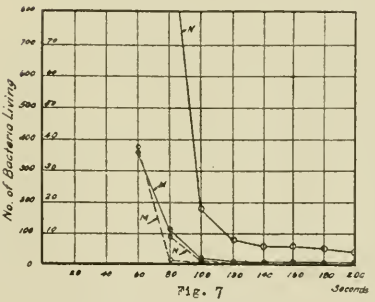

Fig. 7

——— Fluorescent bacteria No. 7

– – – – B.coli communis

—·—·—

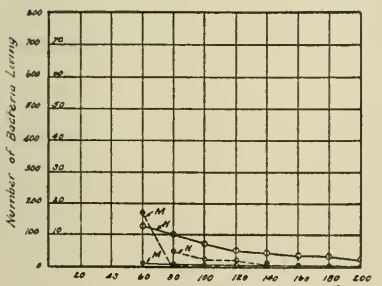

Fig. 6.

——— Fluorescent bacteria No. 6

– – – – Micrococcus capsulatus

—·—·—

seconds. Each curve marked N is plotted to a scale whose ordinate is 10 times as great as that marked M.

It may be seen from the curves that the non-fluorescent bacteria are killed more rapidly than the fluorescent bacteria. Of the non-fluorescent bacteria B coli was the least resistant to the action of ultra-violet, being killed after an exposure of 100 seconds, while B. subtilis was the mst resistant, being killed after 180 seconds. No attempt was made to determine how long an exposure was necessary to kill all the bacteria in any of the cultures of the fluorescent bacteria.

The questions of most interest to us were why are bacteria killed by ultra violet and why are the fluorescent bacteria more resistant than the non-fluorescent. Dreyer, Hanssen[1] and others found that the ordinary proteins such as serum albumin, serum globulin, egg albumin and egg globulin, etc., are comparatively easy to coagulate by ultra-violet. In view of this observation it seemed to us that the most plausible explanation for the bactericidal action of ultra-violet is that the short waves coagulate the protein or protoplasm of the bacteria and by this means kill them. This assumption while explaining the bactericdical action of the short waves does not, however, explain the fact that fluorescent bacteria are more resistant to the action of ultra-violet than the non-fluorescent.

Von Graefe and Helmholtz[2] showed that the lens possesses the property, fluorescence, by which it converts the absorbed short waves into longer waves and gives them off in this form. Burge,[3] while carrying out experiments in an attempt to determine the part played by ultra-violet in the production of glass blower's cataract, found that the lens protein differs from most of the other proteins in that it is very difficult to coagulate by means of ultra-violet. He also showed that the lens protein could be modified by the salts of calcium and magnesium and by sodium silicate, substances found to be greatly increased in human cataractous lenses, so that the short wave-lengths of the spectrum could coagulate it and hence produce an opacity of the lens or cataract. He found that these salts decrease the fluorescence of the lens at the same time that they render the lens protein easily coagu-

[1] Comptes Rendus, 1907, cxiv, 234.

[2] Von Graefe and Helmholtz: Ueber die Fluoreszenz der retina. Poggendorff Annalen der Physik und Chemie, Bd. xciv

[3] Burge: This Journal, 1914, xxxvi.
Hanssen: Comptes Rendus, 1907, cxiv, 234.

lable by ultra-violet. This last observation suggested that there might be some relation between the fluorescence of the lens and its great resistance to the coagulative effect of ultra-violet. To explain the great resistance of the lens protein the assumption was made that the lens by converting the short waves into longer waves and giving them off as such disposes of the energy of the absorbed short waves, which otherwise would have been spent in coagulating its protein. The fact that these substances which render the lens protein more easily coagulable by ultra-violet at the same time decrease the fluorescence of the lens would seem to lend support to this hypothesis.

To explain the great resistance of fluorescent bacteria to the action of ultra-violet an assumption is made similar to that made in explaining the resistance of the lens protein to the action of ultra-violet, namely that fluorescent bacteria protect themselves from the coagulative effect of ultra-violet by converting the short wave-lengths into longer waves and hence dispose of the energy of the absorbed short waves. The non-fluorescent bacteria, however, are unable to dispose of the energy of the absorbed short waves and for this reason they are killed more easily by ultra-violet than the fluorescent bacteria.

THE EFFECTS OF CHANGE IN AURICULAR TONE AND AMPLITUDE OF AURICULAR SYSTOLE ON VENTRICULAR OUTPUT

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What we know concerning the role of the auricles in the dynamics of the heart has been largely a matter of inference: the inference being that the auricles have a passive and an active role; acting passively as reservoirs for the accumulation of blood which is to pass into the ventricles, and actively as pumps, injecting blood into the ventricles and producing a better ventricular filling. The importance of good ventricular filling is apparent when we consider that the ventricle, within certain limits, tends to expel with each systole most of the blood it receives in the preceding diastole. The auricles regulate indirectly by their contraction, the volume output of the ventricles.

To what extent blood pressure and ventricular output are affected by auricular systole has not been a subject of much experimentation. Henderson (1) using the cardioplethysmograph on the heart of the dog, came to the conclusion that auricular systole had practically no filling effect on the ventricles.

With the use of another method, I showed (2) that auricular systole occurring at the normal time interval in the cardiac cycle, increased the blood pressure by 55 per cent of that maintained by the filling action of venous pressure alone. While the filling effect of auricular systole on the ventricles was not directly recorded, it seemed a logical inference, from the experiments then performed, that the increased ventricular output was due largely to an increased ventricular filling. However, at the time, it was considered probable that auricular systole affected ventricular output in other ways. Also it was argued

How many factors are there at work in the interval of auricular systole under consideration causing these large blood pressure changes. Whether the relative amounts of blood forced into the ventricles, and the varied perfection of valvular action alone can account for the changes, is very difficult to say.

The physiological condition of the ventricular muscle at the moment of ventricular stimulation may be of considerable importance. It is a well known fact that a properly stretched muscle gives a far more efficient contraction to a given stimulus than a relaxed muscle. The same probably holds true with ventricular muscle, without in any way being at variance with the 'all or none law.' Straub's records clearly show that the tension of the ventricles is markedly increased by auricular systole, and lasts a short but appreciable time. This period of tension may be of considerable importance. Probably that auricular systole producing it just at the moment the ventricular impulse reaches the ventricles is the most efficient systole in producing the largest end result, namely, ventricular output.

It was to determine the relative importance of increased ventricular filling, perfection of valvular action, and presystolic intraventricular pressure, on ventricular output, and also to determine if other factors are at work, that this research and others concerning the role of auricular systole were undertaken. The experiments reported in this paper have primarily to do with ventricular filling and valvular action.

For purposes of comparison, the method used in the previous work will be briefly reviewed.

The heart of the dog was exposed and independence of the auricles and ventricles established by crushing the auricular ventricular bundle with the Erlanger heart clamp. Arterial and venous pressures, and contractions of the auricles and ventricles were recorded. The ventricles were stimulated at approximately the rate of auricular contraction. By such stimulation "interference waves" similar to interference waves of sound, were produced. That is, the auricles and ventricles at times would beat in accord and at other times in varying degrees of interference. Auricular systole was placed in different phases of ventricular cycle; at one time occuring during ventricular systole, another time early in ventricular diastole, and at another time immediately before ventricular systole.

The method showed that the effects exerted by auricular systole on increasing ventricular output vary with the place occupied by auricular systole in ventricular cycle. The auricular systole placed at about the normal time interval in the normal heart cycle was found to be most effective. In these experiments the magnitude of auricular systole remained constant, the varying factor was time—the time interval at which auricular systole occurred in ventricular cycle.

In the present method, for a particular reason the heart of the river terrapin was used. The auricles of that heart show two distinct types of contraction, the rapid clonic and the slow tonic. The clonic are superimposed upon the tonic, and, as a rule, vary in amplitude, inversely

as the height of the tonic contraction. These properties of the auricles allow the studying of the effect of auricular systole on cardiac dynamics in an entirely different way from that of the previous method mentioned. It permits a study in which the magnitude of auricular systole is the varying factor, while the time interval at which auricular systole occurs in ventricular cycle is the constant factor—the direct opposite of conditions prevailing in the experiments in Method I. In Method I, the possibility of variation in the perfection of valvular action, until eliminated, might be considered a possible controlling factor. In Method II, where auricular systole precedes ventricular systole by the same constant time interval, this possibility is reduced to a minimum.

With changes in auricular tone, the auricular output varies greatly. Therefore, it was thought that if ventricular output varied as a result of auricular tone changes, these variations could be ascribed primarily to different filling effects of the auricles on the ventricles, rather than to variations in valvular action.

To determine whether ventricular output varied with auricular tone, the method represented in the schema was employed.

The heart was perfused in situ with Ringer's solution. The contractions of both auricles were recorded and the ventricular output measured directly. The left Cuvierien duct and hepatic veins were ligated and a cannula of maximum capacity was inserted in the right Cuvierien duct. This cannula was connected with a Marriotte bottle which delivered the Ringer's solution to the base of the heart at any constant pressure. All the arteries coming from the truncus arteriosus with the exception of one were ligated. This artery was connected by cannula with a differential volume recorder.

This recorder consists of a three-way connecting tube with two long vertical arms running upward and one short one running downward. The short vertical tube is supplied with a stop cock. Into one of the long vertical tubes the volume flow to be measured is led. In the other tube is a cork float carrying a glass writing point which records the height of the liquid in the recorder. The stopcock is so regulated that the mean volume flow when led into the recorder will maintain the level of the liquid at about the center of the tube. When the volume flow decreases, the outflow is greater than the inflow, the level of the liquid falls to a point where its pressure will reduce the recorder outflow to the recorder inflow and maintain the writing point at a lower level. When the volume flow increases, the reverse

occurs: the liquid rises until by its increased height it produces a recorder outflow equal to the recorder inflow. The sharpness of the curves indicate the suddenness of change in volume flow.

Without exception all the hearts experimented upon showed marked oscillations of auricular tone. The frequency of the waves varied with the animal. In some experiments in which oscillations were infrequent additional ones were artificially produced either by mechanical irritation (tug on the auricular suspension), or by flooding with a few ccm. of cold Ringer's solution. The results in all cases were the same.

Numerous records were made with venous pressures varying from 10–60 mm. of water. Tone waves occurring with all these pressures had an effect on ventricular output. When the higher venous pressures were employed, however, the tone waves were not nearly as marked, and changes in ventricular output accompanying auricular tonus changes were correspondingly less marked. With venous pressure at about 25 mm. of water the tone waves were most marked and consequently with this pressure the effects of varying amplitude of auricular systole were best studied. This pressure, apparently, permitted heart action as normally seen in the exposed heart of the turtle.

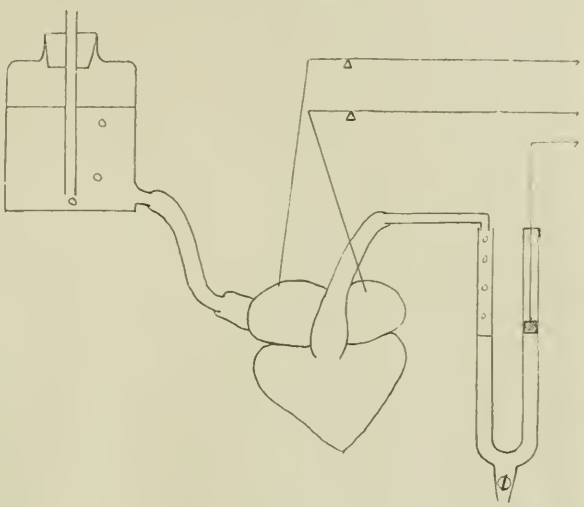

Fig. 1.

Figures 2 and 3 show the usual effects of changes in auricular tone on ventricular output. The corresponding points of the curves are marked. In the case of figure 1 the decreased ventricular output occurring with increase of tone is large. Before the onset of a tone wave, the output was 31 ccm. per minute. This decreased to 10 ccm. per minute. The decrease in ventricular output occurs immediately with the onset of the tonic contraction of the auricles, but becomes more

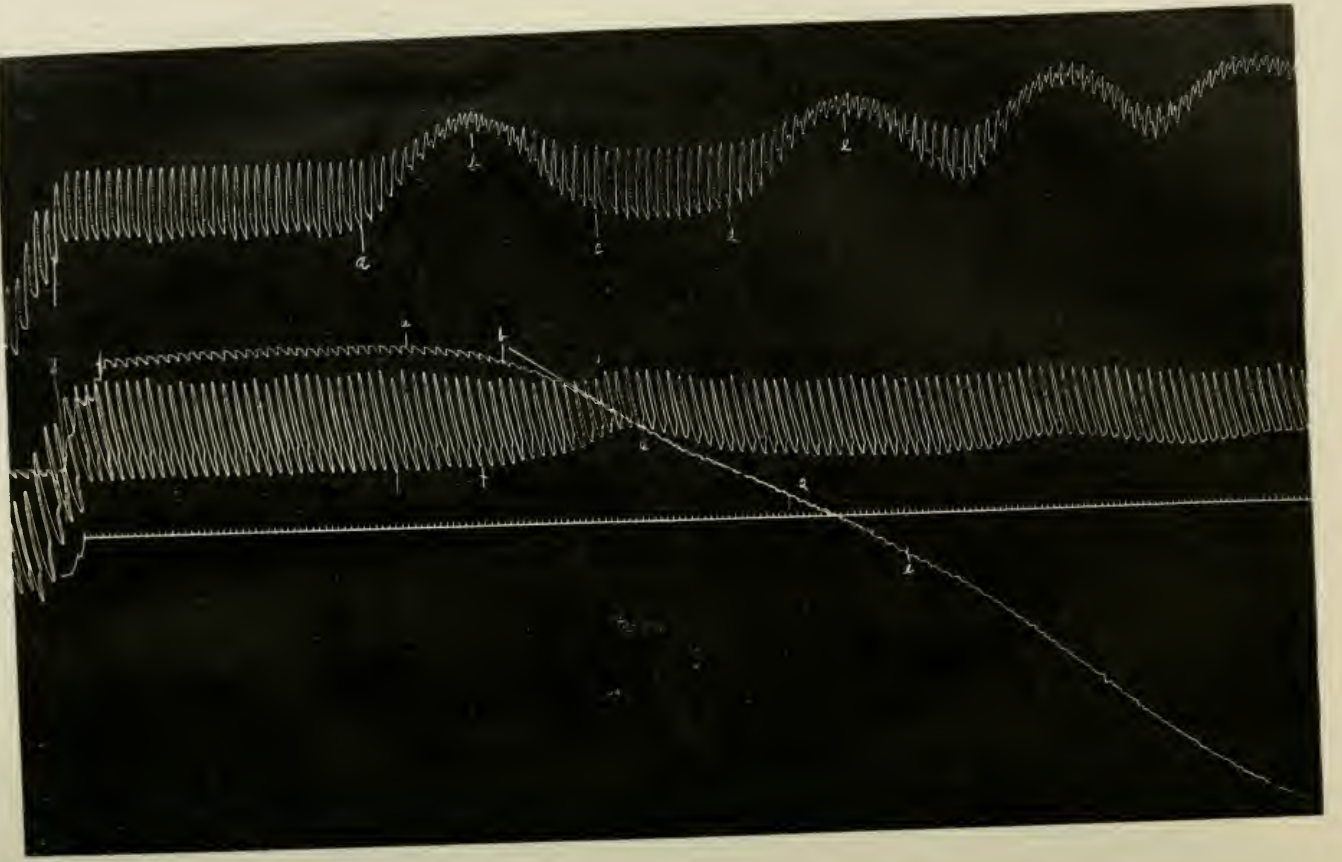

Fig. 2.

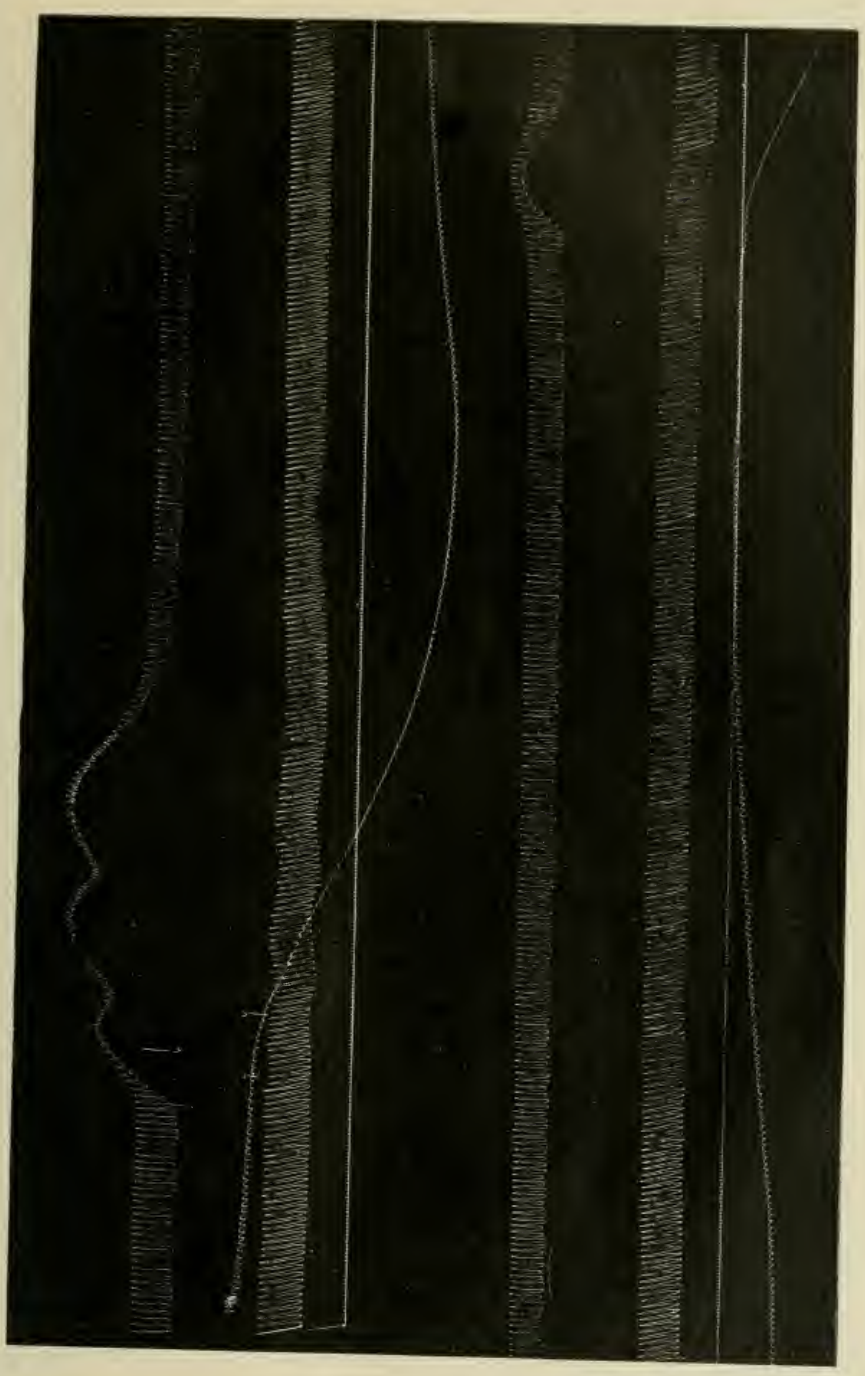

Fig. 3.

marked at the crest of the tonic contraction. The late appearance of the marked decrease undoubtedly is due to the masking effect of the tonic contraction itself. The tonic contraction is fairly rapid, and the filling effect resulting from it astonishingly effective. In some cases where the tonic contractions are exceptionally large, the volume output of the ventricles may be increased during the period of shortening of the tonic contraction, even though the amplitude of the individual auricular systoles are progressively decreasing: the decreased amplitude of auricular systole is largely compensated by the effectiveness of the tonic contraction. The marked decrease of ventricular output occurs where the tonic contraction has reached its crest and the ventricles are filled only by venous pressure and small auricular systoles.

Figure 3 shows the decreased volume output followed by partial recovery. The early compensatory effect of the tonic contraction for the decreasing amplitude of auricular systole is shown again. After the completion of the first part of the tonic contraction, which is the most effective, the volume output decreases rapidly and begins to increase again only with the relaxation of the tonic contraction and increase in amplitude of auricular systole. The increase in volume flow is very gradual, corresponding with the gradual increase in the amplitude of auricular systole. The volume flow never reaches the initial volume output for two reasons: the initial auricular amplitude does not return and the auricle remains in higher tone, consequently having a smaller capacity, which in turn reduces the auricular output. The objection might be raised that the reason for the rapid decrease in ventricular output occurring during the relaxation phase of the tonic contraction is to be found in a diminished venous pressure at the base of the ventricle caused by blood accumulating in the easily dilated auricles. With this objection in mind, a cannula which could deliver from 10-30 times the ventricular output was inserted into the right Cuvierien duct. The very gradual return of volume output occurring with the gradual return of initial auricular amplitude in figure 2 speaks against this objection.

In order to be in a position to ascribe changes in ventricular output to accompanying changes in auricular tone as due purely to an auricular effect, it is necessary to demonstrate that the ventricle itself is not participating in the tone changes. Measurements of the length of ventricular fiber with a myocardiograph were not made, because of the danger of interfering with ventricular contraction and because rhythmical variation in length of ventricular fiber occurring synchron-

ously with oscillations of auricular tone would not necessarily be an index to *true* tonus changes, but rather to differences in the filling of the ventricle occurring during various stages of auricular tone oscillation.

From the experiments I performed, it seemed reasonably safe to assume that true ventricular tone changes did not accompany or follow tone changes in the auricles. The number of tone oscillations in the right auricle were much greater than those in the left. The right and left auricles though much more intimately connected with each other than the auricles and ventricles, seldom showed synchronous tone oscillations. When the oscillations were synchronous, one could not feel sure whether the wave was conducted from one auricle to the other or whether it occurred independently in both auricles.

Rosenzweig (3) states that tonus waves *may* occur synchronously in both auricles and in auricles and ventricles. Fano (4), however, in 100 experiments found the ventricle to show tone oscillation in only three cases.

In view of these facts, and of the fact that ventricular output invariably is affected by changes in auricle tone, it would seem fair to ascribe these changes in ventricular output to auricular tone changes.

Henderson and Johnson (5) in their discussion of valve closure by back pressure and broken jet, state that my previous results may be adequately explained by variation in perfection of valvular action, the valve at times closing by hinge movement from back pressure with considerable regurgitation of blood. This explanation of my results loses much of its force in the present experiments where auricular systole precedes every ventricular systole by the same time interval, thereby exerting its broken jet action on valve closure at all times at the proper moment.

The auricular effects in these experiments apparently are not due to variations of valvular action but to:

1. Variation of auricular diastolic volume. This is a factor of prime importance since it permits a varying auricular output, provided the auricles tend to empty themselves with every contraction and do not meet with materially increased resistance when the auricular output is large than when it is small.

2. Variation in the ability to develop tension in varying phases of tonus oscillations. Patterson, Piper and Starling (6) place considerable importance on the relation between the initial length of mammalian ventricular fiber and tension developed in ventricular systole. If the auricle of the turtle shows the same relation, the auricular sys-

tole occurring when the auricular tone is low should be able to overcome more tension and fill the ventricle better. (I am at present investigating this question.)

1 and 2 would work in the same direction by producing a better filling of the ventricle and in turn an increased efficiency of ventricular fiber.

If presystolic intraventricular tension is of any importance, this factor would vary inversely with the height of tone and work in the same direction as the other auricular effects.

SUMMARY

The effects of variation of amplitude of auricular systole on ventricular output were studied on the heart of the river terrapin.

Because of the oscillations of auricular tone, this heart is peculiarly adapted to this research.

Perfusion of the heart with a constant venous pressure showed variations in ventricular output resulting from oscillations of auricular tone.

Reasons are given for considering variation of valvular action as a minor or negligible factor.

The variations in ventricular output are attributed mainly to the direct effects of variations in ventricular filling.

The slow tonic contractions as well as the rapid clonic contractions of the auricles exert a filling effect on the ventricle in proportion to the amplitude of these contractions.

The decreasing effect of auricular systole of decreasing amplitude occurring during the shortening phase of the tonic contraction is masked by the filling effect of the tonic contraction.

The increased ventricular output resulting from auricular systole of large amplitude is discussed and attributed to

a. Large auricular content at the disposal of auricular systole, this tending to increase the auricular output or ventricular filling.

b. Greater length of auricular fiber, this increasing the efficiency of the auricular contraction and therefore the volume of auricular output, and to

c. Greater ability to increase the length and tension of the ventricular fiber, thereby producing a greater efficiency of ventricular systole.

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STUDIES IN EXPERIMENTAL GLYCOSURIA

IX. The Level of the Blood-Sugar in the Dog Under Laboratory Conditions

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It is a well-established fact that the anaesthetizing and preparation of laboratory animals causes a varying and often very considerable degree of hyperglycaemia to develop. Although there can be no doubt that the influence of certain factors on the blood-sugar level will be masked by the presence of this condition, yet it has so far been impossible to obviate it by procedures which are not open to objections that are probably as serious as the presence of the excess of sugar in the blood.

Both E. L. Scott (1) and Shaffer (2) have recently shown that the blood-sugar level in the dog and cat is really much below that usually taken as the 'normal' in experiments bearing on sugar metabolism. Scott concludes that "if consistent results are to be expected, the animals (cats) must be . . . . killed without pain or excitement," and Shaffer, working on the dog, shows that the removal of blood from a vein with entire avoidance of any excitement on the part of the animal, yields blood-sugar values which are about one-half those which have been considered by previous workers as normal. Both investigators emphasize the diabetic influence of the excitement caused by giving a general anaesthetic, and in a later communication, Shaffer (along with Hubbard (3)) shows that the initial hyperglycaemia may be avoided, or, if present, caused almost to disappear by forced respiration through a tracheal cannula.

Similar observations by other investigators are referred to fully in the above papers.

If we are to confine our investigations on the behaviour of the sugar level of the blood to unanaesthetized animals, however, a very limited field will be open to us, for we shall be unable to study many of the factors which influence the glycogenic functions of the liver. For purposes of following the blood-sugar level from day to day, the precautions advocated by the above workers must henceforth be strictly adhered to, but for the large group of observations requiring surgical interference, and therefore anaesthesia, an entirely different method must be followed in obtaining normal standards with which to compare the experimental results. Shaffer and Hubbard suggest that in such cases the initial hyperglycaemia, since it is largely due to dyspnoea, should be avoided by forced artificial respiration. One of us (J. J. R. M.) (4) some years ago employed this method to avoid the dyspnoeic hyperglycaemia brought about in dogs by stimulation of the central end of the vagus nerve. It was also used in experiments involving stimulation of the splanchnic nerve. The forced ventilation was always found to cause a decline in blood-sugar, but the method was abandoned as a routine practice in subsequent work because it was concluded that the depression in the CO_2-tension of the blood involved in using it would introduce conditions which were at least as unphysiological as those due to a slight excess of sugar. We have frequently reconsidered the advisability of using forced respiration or insufflation of oxygen in our experiments, but as work has accumulated showing, on the one hand, the close relationship between the CO_2-tension of the blood and its reaction and, on the other, the susceptibility of the physiological activities of nerve centers, as well as of many organs and tissues—including almost certainly the glycogenic function of the liver—to the reaction of the blood, we have abandoned the idea.

These difficulties in securing constantly low 'normal' values for blood-sugar in laboratory experiments make it necessary to adopt other standards with which to compare the results obtained during some experimental procedures. The ideal being unattainable at present, there remains available one of two methods, either: (1) to determine the blood-sugar level of each animal for some time before bringing about any experimental change, or (2) to use, for comparison with the experimental animal, data secured from a sufficient number of anaesthetized animals in which all the conditions are as similar as possible to those obtaining in the experimental animal.

We have hitherto employed the former of these methods although, as already stated, we have recognized the limitations due to the fact that the initial disturbances dependent upon etherization, etc., might mask many of the results. For further work which we contemplate, however, it will be necessary to employ the second method, and some of the data which we have collected to this end are given in the present paper.

Methods. Dogs were employed in all the experiments. Most of these were removed from the stock kennels several days before being used and were either starved or given a liberal diet of bread broken up in a meat broth. Certain of the animals were given 5–7 grams of cane sugar per kg. body weight, dissolved in water, by stomach tube, on the evening preceding the experiment. Etherization was brought about as quickly as possible, and the animal was then tied out on a warmed operating table and a tracheal cannula introduced and connected with an anaesthetic bottle. Throughout the remainder of the experiment the concentration of ether in the inspired air was kept as constant as possible. The arterial blood pressure, the respiration and the rectal temperature were carefully watched.

After opening the abdomen the aorta was ligated between the coeliac axis and renal arteries, and the vena cava tied at the same level. A cannula was then introduced in the vena cava so that its open end lay opposite the hepatic veins. This cannula was plugged by a pipe cleaner. To secure samples of blood from the portal vein a similar cannula was inserted in the pancreatico-duodenal vein with its free end just at the vena porta. For the analyses samples of blood were removed, usually at intervals of two minutes, by removing the pipe cleaners and connecting a pipette to the cannula. A sufficient amount of blood was removed to be certain that the cannula was filled with that present in the particular vein under observation. This pipette was then removed and a moistened 2 cc. pipette connected with the cannula. There was practically never any trouble with clotting, for if a clot did form it remained adherent to the cleaners and was withdrawn with them.

The 2 cc. samples of blood were immediately transferred to test tubes containing 8 cc. water, and the sugar content was ascertained by one of the methods described elsewhere by one of us (R. G. P.) (5).

Portions of liver were also removed at the end of the experiments for glycogen estimation.

Results. The following features of the blood-sugar level have occupied our attention in the present investigation, viz: (1) the extent of the fluctuations occurring under normal conditions; (2) the initial height of

the level in relationship to the amount of glycogen in the liver; (3) the relationship between the levels in the blood of the portal vein and vena cava.

Although we have performed a considerable number of experiments, we do not consider that we have by any means sufficient data from which

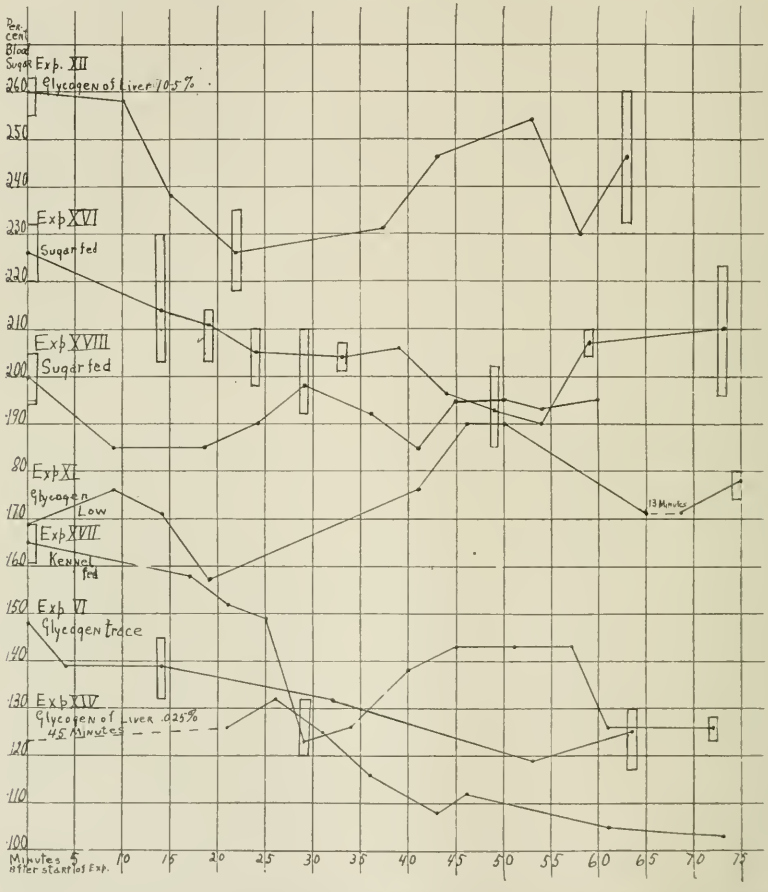

Fig. 1. Curves from seven experiments showing the fluctuations in the sugar level in the blood of the vena cava.

all of these questions can be finally answered, but we have thought it advisable to place what we have on record.

The extent of the fluctuations. For most of the experiments in this connection the modified method of Bang was employed, as described

by one of us (R. G. P.) elsewhere, and the results of seven of them are compiled as curves in figure 1. The extent of the experimental error involved in this method, as indicated here and there along the curves by vertical oblongs, is seen to be considerable. Usually, however, it is below 5 per cent, and frequently duplicate analyses agreed exactly. After allowing for the greatest possible error, it is plain that considerable fluctuations occur, even when the two specimens of blood were removed at intervals of five minutes apart. It will be noticed that the fluctuations are of two types, one, sudden, occurring over a period of a few minutes, and the other, much more gradual.

A. *The sudden fluctuations.* It is probable that these are often due to experimental error, but this is not always the case because they are also present when the much more accurate modified Benedict method is employed. The results of three experiments of the same type in which the estimations were made by this method, are compiled in the curves of figure 2, from which it will be observed that the samples of blood were removed every two or three minutes, instead of every five. This permits us to determine with greater certainty whether these momentary fluctuations actually exist, which, it will be seen, they do, although usually very slight in degree. Thus, in the fifty or so estimations used in the compilation of the curves, the greatest fluctuations occurring in two minutes are a fall from 0.156 to 0.137 per cent (No. 78) and a rise from 0.140 to 0.160 per cent (No. 76). Earlier in this latter experiment two consecutive rises of about 15 per cent each are also present. The former variation occurred immediately after the start of the experiment, and is probably to be attributed to operative

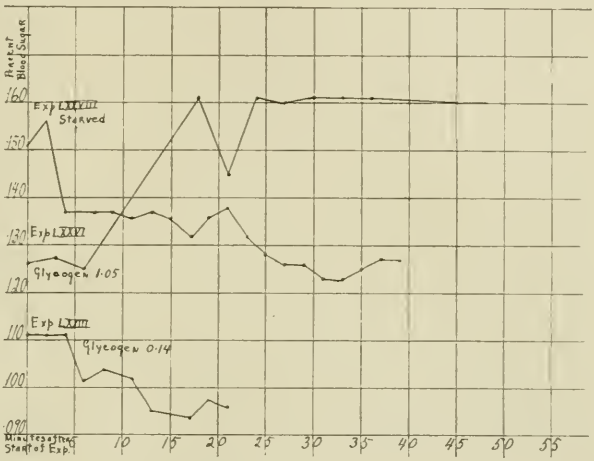

Fig. 2. Curves showing behavior of blood-sugar level compiled from data furnished by a modified Lewis-Benedict method.

disturbances involving the portal circulation. We have observed similar declines in the blood-sugar during the first few minutes of other experiments of this type, so that we always allow a sufficient time for it to occur before attempting to bring about any experimental change. The sudden rises seen in experiment 76 are more serious and we are at a loss to explain them. The animal used in the experiment behaved under anaesthesia in a perfectly normal fashion, and there can be no doubt about the accuracy of the estimations, since blood samples taken from the portal vein gave values which agreed very closely with those taken simultaneously from the vena cava. Unusual though such sudden fluctuations may be, it is essential that their possible occurrence in work of this nature should be allowed for by frequent repetition of the experiments.

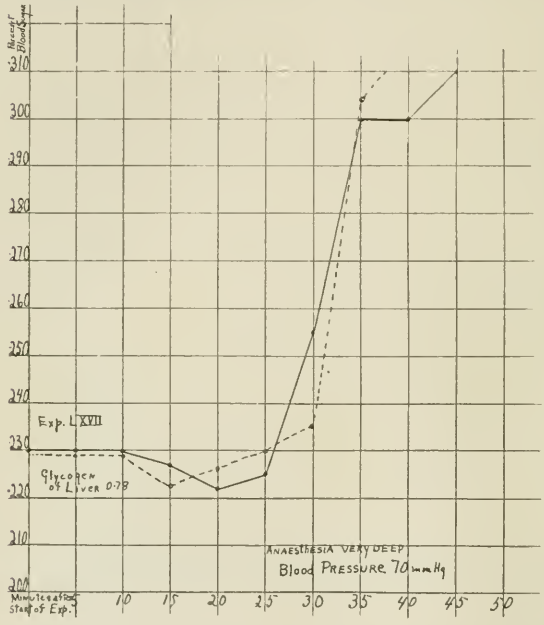

Fig. 3. Curve from experiment in which a sudden fall in blood pressure occurred following a period of faulty anaesthesia.

Such sudden rises in the blood-sugar level occurring unexpectedly during the course of an experiment on an apparently 'normal' animal should be distinguished from similar rises almost invariably to be observed when the animal becomes asphyxiated, or when there is a pronounced fall in arterial blood pressure. In figure 3 curves plotted from the results of such an experiment are given, the animal being hyperglycaemic from the very start.

B. *The gradual changes.* In all the eleven experiments, save the one already referred to (viz., No. 76), there occurred a progressive fall in the blood-sugar level during the first part of the period of observation. This fall lasted for at least thirty minutes, although it persisted for the

whole duration of the observation in about one-half of the experiments (viz., Nos. 6, 14, 16, 73, 78). The gradual decline is probably due to the passing away of conditions which excite the glycogenolytic process during the anaesthetisation and operative manipulation of the animal.

Such evidence that the preparation of the animal causes hyperglycogenolysis, along with the marked variation in the level to start with, might seem to indicate that investigation of the glycogenic function would be impossible in anaesthetized animals, since the already existent disturbance would mask any further change, especially an increase, which might be brought about by experimental interference. It is very probable that conditions which would cause a slight increase of blood-sugar in a 'normal' animal might fail to do so in one that was hyperglycaemic, but this fact does not, in our estimation, justify the conclusion of one author that 'the dog is little suited for investigations concerning glycaemia' (Bang) (6). The emotional glycaemia is at least as marked in the other animals available for such work (cat and rabbit), and, in the case of the rabbit at least, there are much more serious objections to their employment, such as their small size, their herbivorous habits and the impossibility of removal of the pancreas. Unless dogs are employed, investigation of many of the problems concerning the glycogenic function of the liver becomes impossible, but to discount the disturbing influences due to etherization and operative interference we must know exactly the extent and frequency of such disturbance.

The initial level and the subsequent rise in blood-sugar in relationship to the percentage of glycogen in the liver. It is clear from figures 1 and 2 that an initially high level of blood-sugar is more likely to occur in animals having a high percentage of glycogen than in those in which there is only a trace. The secondary rise is also usually more marked in glycogen-rich animals, although it sometimes occurs, as in experiment 11, in those that are glycogen-poor.

The relationship between glycogen-content and the initial sugar-level is well illustrated in the following table (I) taken from other experiments than those used in the compilation of the curves in figure 1.

The relationship between the levels in the blood of the portal vein and vena cava. On account of the magnitude of the blood flow through the liver, the very smallest difference in sugar concentration in the blood entering and leaving it could exist only when an extreme degree of glycogen formation or breakdown existed in the liver. In a subsequent paper we shall submit results showing how such 'percentage' values behave when dextrose is injected into the portal vein. In the present

paper we have collected, in Table II, the results of estimations which were made on samples of blood which were collected from the two vessels either simultaneously or at intervals of not more than one minute apart and prior to the sugar injections.

TABLE I

Relationship between the initial sugar-level and the glycogen-content of the liver.

STARVED			WELL-FED		
Number of experiment	Glycogen in liver	Sugar in blood at start	Number of experiment	Glycogen in liver	Sugar in blood at start
	per cent	*per cent*		*per cent*	*per cent*
31	trace	0.176	32	6.50	0.246
36	trace	0.098	34	0.70	0.163
38	trace	0.090	35	3.62	0.161
41	trace	0.110	37	5.70	0.181
44	trace	0.085	40	1.57	0.200
45	trace	0.075	42	10.37	0.202
49	trace	0.131	47	0.41	0.170
50	trace	0.120	46	9.12	0.118
51	trace	0.118	48	2.00	0.122
65	trace	0.168	53	12.00	0.170
70	trace	0.146	54	4.16	0.205
63	trace	0.130	62		0.150
			68	0.67	0.100
			72	6.5	0.200
	Max mum	0.176		Maximum	0.246
	Minimum	0.075		Minimum	0.100
	Average	0.120		Average	0.170

TABLE II

NUMBER OF EXPERIMENT	SUGAR IN BLOOD OF PANCREATICODUODENAL VEIN	SUGAR IN BLOOD OF VENA CAVA	INTERVAL BETWEEN COLLECTION OF SAMPLES OF BLOOD	
			(1) From same vein	(2) From other vein
	per cent	*per cent*	*min.*	*min.*
65	0.170, 0.168	0.168, 0.167	1	1
70	0.133, 0.133	— , 0.146	1	1
68	0.100, 0.099, 0.100	0.100, 0.098, 0.100	1	1
71	0.137, 0.136, 0.126	0.140, 0.140, 0.140	2	0.5
72	0.200,	0.200, 0.207	2	0.5
74	0.160, 0.148, 0.160	0.160, 0.160, 0.160	2	0.5
75	0.158, 0.138	0.166, 0.158, 0.158	2	0.5

A remarkable correspondence is evident between the two bloods in most cases. When any difference exists it is always small in degree and is, with one exception (No. 70), due to a decline in the portal blood value (Nos. 71, 74, and 75). In the case of No. 74 it is possible that the value 0.148 is due to experimental error, but we have no reason to suspect this in the two other experiments in which the decline occurs (Nos. 71 and 75). In two experiments (Nos. 73 and 76) the estimations were made over longer periods without injecting dextrose into the portal circulation. The results of these experiments are given in the curves in figure 4, from which it will be seen that in one of the experiments (No. 73) there

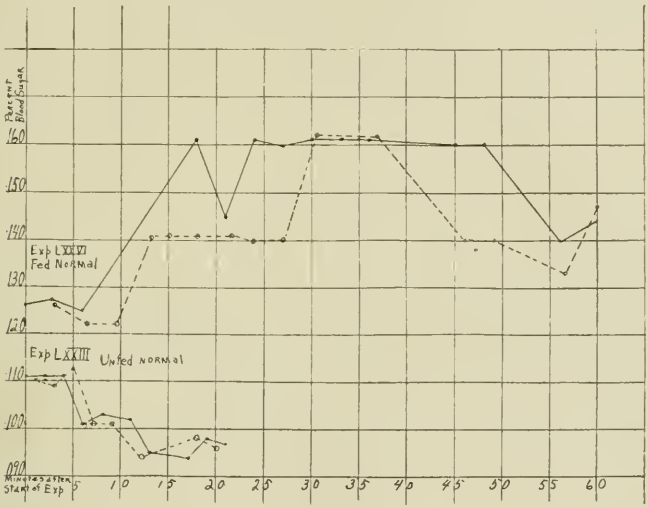

Fig. 4. Curves showing blood-sugar levels in vena cava and portal vein in two dogs which were only anaesthetized.

was entire correspondence, whilst in the other (No. 76) the sugar concentration of the 'cava' blood stood very considerably above that of the portal blood during a period of nearly twenty minutes. This result indicates a high degree of hyperglycogenolysis for which there was no apparent cause. The behaviour of the curve for the portal blood indicates further that the increase of sugar in the blood of the systemic circulation did not in this experiment immediately cause a similar increase in that of the portal vein. The excess of sugar is evidently retained somewhere in the organism, probably in the muscles. In the experiment in which artificial glycaemia was brought about by

injecting dextrose into the portal vein, this delay in the appearance of the excess of dextrose in the portal blood was not noticed (see subsequent communication). We cannot account for it in the present experiment unless we assume that dextrose which has been set free in the liver as a result of hyperglycogenolysis is more liable to be 'fixed' or used in the tissues than injected dextrose. Although there is so far no direct evidence (7) that glycogen-dextrose is dealt with by the tissues in any different manner from chemically pure dextrose, yet it is possible that it is so, and that one of the differences is with regard to its readiness to become deposited in the tissues.

In conclusion we would point out that although the blood-sugar level in an anaesthetised animal does not remain unchanged from time to time, yet the changes occurring during a period of ten minutes are very small when compared with those observed by us on blood similarly removed from animals in which the splanchnic or hepatic nerves were stimulated (8).

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STUDIES IN EXPERIMENTAL GLYCOSURIA

X. The Sugar Retaining Power of the Liver in Relationship to the Amount of Glycogen Already Present in the Organ

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Since the time of Claude Bernard it has been usual to consider the glycogenic function of the liver as one analogous to that of starch formation in plants, that is, as a means for temporarily storing away for future uses in the organism, carbohydrate that is not immediately required. It has been recognized, however, that the capacity of the liver to store glycogen is quite inadequate to account for all the carbohydrate that is assimilated from the intestine, and it is believed that some of the excess is carried to the muscles and deposited there as glycogen, the remainder being either oxidized or more slowly converted into fat. According to the above view, we should expect that the power of the liver to retain sugar as glycogen would be more marked when this organ is empty than when it is already well filled with glycogen. Undoubtedly, such a view is correct when we consider the *total glycogen capacity;* that is to say, the empty liver will hold more than the liver that is already partly filled. But this does not necessarily mean that the *rate* with which the liver converts the sugar to glycogen will be different in the two cases.

Capacity for glycogen and rate of glycogen-formation in the liver need not run parallel. Indeed there are several observations on record which would seem to show that dextrose, absorbed from the intestine, passes more freely into the blood of the systemic circulation in fasting, than in well-fed animals. Thus, Bang (1) and his collaborators have found that after giving from 2 to 10 grams of dextrose by stomach to rabbits, the dextrose concentration of the systemic blood began to rise in fifteen minutes, gaining a maximum in about an hour and then returning to the normal level in three hours. This hyperglycaemia de-

veloped more rapidly and reached a higher level in starved rabbits than in those that had received previous administrations of dextrose; and it usually failed to develop at all in animals that had received and recovered from several previous injections.

Such a result can most easily be interpreted by assuming that the liver more quickly removes the absorbed dextrose when it already contains some glycogen than when it is glycogen-free. If these results can be confirmed, it would indicate that the process which is going on in the liver during starvation is one of glycogenolysis, or more correctly of glyconeogenesis, and that the liver cell cannot immediately reverse it to one of glycogen-formation (glycogenesis) when excess of dextrose appears in the portal blood. The liver cells, in other words, cannot produce glycogen and dextrose at the same time, either when the dextrose comes from their own stores of glycogen or from protein, and it takes some time for the cells to change over from the one function to the other.

The present investigation was undertaken to supply more direct evidence for or against the above hypothesis.

Methods: Dogs were either starved or fed for some days on a diet of bread and meat, or given cane sugar by stomach tube on the evening preceding the experiment. After being anaesthetized with ether, cannulae were placed in the inferior vena cava and, in the later experiments, in the duodenal vein, as described in a previous paper. A cannula filled with Ringer's solution was also placed in one of the branches of the mesenteric vein, as far down as possible. Through the tubing which closed this cannula passed a hypodermic needle connected through a three-way stop-cock with tubes leading to the bottom of two graduated cylinders, one containing Locke's solution, the other, a 4 per cent solution of pure dextrose. The graduates were closed above with stoppers, through a second hole in which they were connected with an 8-litre bottle, in which an air pressure was established by means of a pump. The large capacity of the bottle and the comparatively slow rate of outflow through the hypodermic needle ensured a practically uniform rate of injection during five minutes, and this rate was altered in the different experiments, either by choosing needles of different bore or by changing the air pressure—by means of a mercury valve—in the bottle. Although somewhat hypotonic, the above strength of dextrose was chosen to simplify calculation, since we intended, at the outset, to alter the rate of injection by known degrees during each experiment. The rate of injection varied from 8 to 27.5 cc. in the different experi-

ments, i.e., from 0.32 to 1.2 grams of dextrose in five minutes. Taking the average blood-flow through the portal vein as 1300 cc. in five minutes (calculated on the basis of 4.3 cc. per second for a liver of average weight, 400 grams) (2), and assuming that the blood to start with contained 0.15 per cent dextrose, the above amounts of injected dextrose would give a maximal percentage of 0.25 in the portal blood, supposing none of the dextrose was removed from the blood during the five minutes of injection. This degree of hyperglycaemia in the blood of the portal vein is believed to be not infrequently overstepped during the absorption of carbohydrate, so that the conditions in our experiments in this regard can be considered as well within the normal limits.

In order to ascertain the normal sugar concentration of the blood of each animal, several samples of blood (2 cc. each) were removed at two-minute intervals from the vena cava, and also—in the later experiments —from the duodenal vein. Meanwhile, in most of the experiments, Locke's solution was being injected into the mesenteric vein. The sugar solution was then injected for exactly five minutes, samples of blood being still collected every two or three minutes, and at similar intervals after discontinuing the injection. A second injection period followed after from ten to twenty minutes, unless the condition of the animal was such as to make it inadvisable.

At the end of the experiment a portion of liver was removed and cut in thin slices, which were then dried between filter paper. Duplicate portions, of 10 grams each, were then used to determine the glycogen-content. In some of the earlier experiments a piece of liver was removed, after mass ligation, early in the experiment. It was hoped that results could be secured, from a comparison of the glycogen-content at the beginning and the end of the experiments, that would furnish information as to whether glycogenesis or glycogenolysis was taking place during the time of the observation. So far, the results in this respect have not been satisfactory. Anaesthesia, arterial blood pressure, and the respirations were practically unchanged during the experiments, unless otherwise noted in the tables of results, which follow.

Discussion of results: These are compiled in Tables I and II. It will be most convenient to consider them as a whole first of all, and later to select one or two typical experiments for the purpose of examining more closely the time relationships and the magnitude of the changes produced by the sugar injections.

The figures in the fourth columns of the tables represent the grams of dextrose injected into a mesenteric vein in five minutes; those of the

TABLE I

The effect of dextrose injection into the portal vein of starved dogs on the dextrose-content of the blood of the vena cava

1 NO.	2 WEIGHT	3 FOOD	4 DEXTROSE INJECTED	5 BLOOD-DEXTROSE BEFORE INJECTION	6 BLOOD-DEXTROSE DURING INJECTION	7 AVERAGE INCREASE	8 INCREASE PER 1 GM. DEXTROSE	9 DO. MULTIPLIED BY THE BODY-WEIGHT	10 MAXIMAL INCREASE DURING (D) OR IMMEDIATELY FOLLOWING (A) INJECTION	11 DIFFERENCE BETWEEN AVERAGE PRECEDING AND MAXIMUM DURING	12 GLYCOGEN IN LIVER	13 REMARKS
	kg.		*gm.*	*per cent*	*per cent*	*per cent*	*per cent*				*per cent*	
31	17.3	Starved	1.00	0.176⊙	0.255※	0.079	0.079	1.36	0.30D	0.084	Trace	⊙Slowly rising. ※Did not come back.
36	21.80	Starved	0.88	0.098	0.106※	0.008*	0.009	0.246	0.127D	0.029	Trace	※Hyperglycaemia following.
36a	21.8	Starved	1.00	0.086	0.117⊙	0.031	0.031	0.676	0.132D	0.046		⊙Came back partly.
38	10.9	Starved	0.72	0.090	0.132	0.042	0.058	0.163	0.158A	0.068	Trace	
38a	10.9	Starved	1.20	0.106	0.112	0.006※	0.005	0.054	0.182D	0.076		※Increased considably after.
41		Starved	0.80	0.110	0.173	0.063*	0.080		0.20D	0.090	Trace	※One estimation.
41a		Starved	0.80	0.104※	0.214	0.110*⊙	0.127		0.24D	0.114		⊙Came back partially
44	8.17	Starved	0.52	0.085	0.097	0.012	0.023	0.189	0.105D	0.020	Trace	
44a	8.17	Starved	0.40	0.083	0.104	0.021	0.052	0.426	0.115D	0.032	Trace	
45	10.00	Starved	0.24	0.075	0.094	0.019*※	0.079	0.179	0.103A	0.028	Trace	※Did not come back. Dog died.
49	19.00	Starved	0.24	0.131	0.157	0.026	0.108	2.052	0.158D	0.028	Trace	
49a	19.00	Starved	0.44	0.130	0.155	0.025	0.057	1.08	0.162D	0.032	Trace	
50	9.50	Starved	0.64	0.120	0.180	0.060	0.093	0.880	0.195D	0.075		
50a	9.50	Starved	0.48	0.125	0.162	0.037	0.077	0.730	0.169D	0.044	Trace	
51	8.65	Starved	0.32	0.118	0.145⊙	0.027*	0.084	0.720	0.164D	0.046		⊙Did not come back till after second injection. Died second injection.
65*	5.20	Starved	0.40	0.168	0.214	0.046*	0.115	0.600	0.248D※	0.080		※P. D. & V. C.
70	22.30	Starved	0.48	0.146⊙	0.146	None			0.158	0.012		⊙One estimation.
71?	6.80	Fed※	0.60	0.140	0.160†	0.020*	0.033	0.22			Trace	※Fed 3 days with special diet; P. D. & V. C. †Partial recovery.
71a	6.80	Fed	0.48	0.184	0.215⊙	0.039	0.081	0.55				⊙One estimation.

TABLE II

The effect of dextrose injections into the portal vein of well-fed dogs on the dextrose content of the blood of the vena cava

1 NO.	2 WEIGHT	3 FOOD	4 DEXTROSE INJECTED	5 BLOOD DEXTROSE BEFORE INJECTION	6 BLOOD DEXTROSE DURING INJECTION	7 AVERAGE INCREASE	8 INCREASE PER 1 GRAM DEXTROSE	9 DO. MULTIPLIED BY THE BODY-WEIGHT	10 MAXIMAL INCREASE DURING (D) OR IMMEDIATELY FOLLOWING (A) INJECTION	11 DIFFERENCE BETWEEN AVERAGE PRECEDING AND MAXIMUM DURING	12 PER CENT GLYCOGEN IN LIVER	13 REMARKS
	kg.		*gram*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*		
32	12.7	Sugar	1.08	0 246	0.265	0.019*	0.017	0.216	0.325A	0.079	6.5	
				0.266	0.284	0.018	0.016	0.203				
34	12.7	Sugar	1.20	0.163⊙	0.194 ⁂	0.031*	0.026	0.330	0.195D	0.032	0.715	⊙Results irregular.
												⁂ Did not come back.
35	16.3	Sugar	0.80	0.161	0.157	None	None		0.162A	0.00	3.62	
37	13.1	Sugar	0.64	0.181	0 217	0.036 ⁂*	0.056	0.734	0.294A	0.113	5.7	⁂ Marked hyperglycaemia steadily developed. *See Curve.*
40	7.27	Sugar	0.64	0.200	0.255	0.055 ⁂*	0.085	0.618	0.260D	0.060	1.57	⁂ Only recovered slightly.
												⊙ 1 estimation.
40a	7.27	Sugar	0.80	0.232⊙	0.316	0.084*†	0.105	0.763	0.344A	0.112	1.57	†Recovered still less.
42	10.9	Sugar	0.80	0.202	0.245	0.043 ⁂*	0.050	0.545	0.254D	0.052	10.37	⁂ No recovery.
42a	10.9	Sugar	0.64	0.253	0.276	0.023 ⁂*	0.030	0.327	0.276D	0.023		
47	10	Sugar	0.80	0.170	0.170	None ⁂	Decrease		0.170D	0.000	0.412	⁂ Really hyperglycaemia.
47a	10	Sugar	0.68	0.164	0.141	Decrease	Decrease		0.141D	0.000		
46	10.9	Sugar	0.72	0.118	0.137	0.019	0.026	0.283	0.151D	0.033	9.12	
46a	10.9	Sugar	0.48	0.115	0.153	0.038	0.078	0.850	0.163D	0.048		
48	18	Sugar	0.48	0.122	0.149⊙	0.027	0.056	1.040	0.260D	0.138	2.00	⊙Very irregular results. Immediate effect hyperglycaemia.
48a	18	Sugar	0.40	0.140	0.231	0.091†	0.23	4.150	0.280D	0.140		†Start later than usual. Not included.
53	9.9	Bread	0.48	0.170	0.176	0.006 ⁂*	0.012	0.12	0.188A	0.018	12.00	⁂ Did not come back.
			0.40	0.235	0.217	Decrease	Decrease					
54	13.5	Bread	0.88	0.205	0.254	0.049*	0.056	0.756	0.262D	0.057	4.16	Altering infusion of Ringer starts here.
			0.96	0.246	0.277	0.031	0.032	0.432	0.316D	0.070		
62	6.5	Bread	0.20	0.15	0.155	0.005*	0.025	0.162	0.165D	0.015		
			0.60	0.169	0.242	0.073 ⁂*	0.120	0.780	0.27D	0.100		⁂ Did not come back.
			0.44	0.26	0.260	No change	No change					
68	14.3	Bread	0.64	0.100	0.112 ⁂	0.012	0.019	0.270	0.118D	0.018	0.675	⁂ Exactly parallel rise in P. V.
72	12.6	Bread	0.48	0.200	0.218 ⁂	0.018	0.0375	0.47	0.220D	0 020	6 5	Hepatic plexus cut.
72a	12.6	Bread	0.48	0.246	0.309⊙	0.063	1.31	1.65	0.392A	0.1		⁂ Came back increasing later.
74	8.7	Bread	0.32	0.160	0.176	0.016*	0.031	0.270	0.192D	0.002		⊙One estimation omitted.
74a	8.7	Bread	0.4	0.176 ⁂	0.191	0.015	0.037	0.322	0.197D	0.021		⁂ One estimation.

*The asterisks indicate that the percentage of sugar did not return to the normal within five minutes after the injection.

fifth, the percentage of dextrose in the blood of the vena cava immediately preceding the sugar injection. In practically every instance these figures, i.e., in column 5, are the average of determinations on several samples of blood removed at two-minute intervals. The sixth column gives the average percentage of blood-sugar during the injection of the sugar, and the seventh, the average increase. This average increase is nearly always considerably less than the maximal increase, which is given in the tenth columns, because the values in the samples of blood obtained immediately after the injection did not, in most cases, show any increase over the normal. On this account, and also because the first samples taken after discontinuing the injection still showed the increase in sugar, it might have been advisable to use, for the computation of the average, not the values obtained during the injection, but rather those immediately preceding and following the end of the injection period. This method was not chosen because it was found in many cases that the increased discharge of sugar was maintained for some considerable time after discontinuing the injection, thus indicating that a hyperglycogenolysis had become established, probably as a result of the sugar injections. This stimulation of a more or less persistent sugar mobilization will be discussed in a future paper; meanwhile it is important to note that it was more frequent in the case of glycogen-laden livers than in those that were glycogen-free, so that, to have used the figures obtained after the injection, would have made the averages for fed animals too high. The cases in which the sugar percentage did not return to the normal within about five minutes after the injection are indicated by asterisks.

Since figures in the seventh columns represent increases produced by the injection of varying amounts of dextrose into the portal vein, they cannot be compared with one another. To make this possible it was necessary to calculate in each case the average increase for 1 gram of injected dextrose. This has been done by direct proportion—figures in column 8—on the assumption that the same fraction of injected sugar will always be retained by the liver, an assumption for which there is however no experimental justification, for it may well be that, with progressively increasing injections, a greater or a smaller fraction of the sugar is retained. We intend to investigate this point at an early date.

To further reduce the figures to a common basis allowance had of course to be made for the greater quantity of blood in the portal circulation in large, as compared with small animals. Since the total volume

of blood in the body is practically proportional to the body weight, we have made this correction by multiplying the figures in the eighth column by those in the second, since the larger the animal, the greater must be the dilution to which the injected sugar would be subjected. The values thus obtained are more or less arbitrary, but they are comparable one with another. The figures in the twelfth column give the percentage amounts of glycogen found present in portions of liver removed immediately after the animal was killed, or, in some cases, in portions removed before the injection was started.

Although the values obtained after making the corrections as above indicated—i.e., those in the ninth column—do not exhibit as close a correspondence as we had hoped they would, yet, when we compare them in Tables I and II, they seem sufficient to justify the general conclusion that the glycogen-free liver does not remove dextrose from the portal blood any more quickly than one that is glycogen-rich. The ultimate storage capacity of the glycogen-free liver is no doubt greater than that of one that is already partly filled with glycogen, but its avidity for dextrose is certainly not more pronounced; indeed, if anything, it appears to be somewhat less, as a close comparison of the results in the two tables will indicate.

It will be noted that the percentage of dextrose in the blood issuing from the liver at the start of the experiment is very much more constant in the case of the starved animals than in those that were fed. The variation for the former is between 0.075 and 0.146 (leaving out the values in experiments 31 and 65, which are considerably higher) and the average 0.111. The variation for the latter is between 0.100 and 0.206 (leaving out experiment 32) and the average 0.150. This is of course what we should expect, and is entirely in line with our previous experience that operative hyperglycaemia is much more likely to develop in well-fed, as compared with starved animals. The differences, however, render comparison somewhat uncertain, and they make it desirable to compare the extent of the hyperglycaemia produced by 1 gram of dextrose in animals having about the *same* initial blood-sugar percentage. This is done in the following table (Table III).

It will be seen that the dextrose tends to pass the liver more readily in the starved group of animals.

The initial hyperglycaemia probably does not introduce any very serious error, for the increase produced by injecting 1 gram of sugar is at least as marked in initially hyperglycaemic, as in normal animals. Thus, for sugar percentages up to 0.16, the increases were: 0.330, 0.00,

TABLE III

STARVED ANIMALS		WELL-FED ANIMALS	
Original percentage of dextrose in blood	Increase in percentage of dextrose per 1 gram dextrose multiplied by the body weight	Original percentage of dextrose in blood	Increase in percentage of dextrose per 1 gram dextrose injected multiplied by the body-weight
0.098	0.246	0.100	0.270
0.090	0.630		
0.118	0.720	0.118	0.280
0.140	0.220	0.150	0.162
		0.160	0.270
		0.161	0.000
		0.163	0.330
0.168	0.600	0.170	0.000
		0.169	0.120

0.000, 0.283, 0.850, 0.162, 0.27, 0.270; and in those having an initial blood-sugar percentage of 0.200 or above, they were: 0.216, 0.203, 0.618, 0.763, 0.545, 0.327, 0.000.

The uncertain nature of the results of the above experiments led us to suspect that the differences obtained might be due to unequal amounts of sugar entering the liver, for although we always examined to see that the sugar solution was really going into the mesenteric vein, yet it might not become properly mixed with the blood by the time that the hilus of the liver was reached. To control this we performed several experiments in which blood was taken from the portal vein, through a cannula inserted in the central end of the pancreatico-duodenal branch, at about the same time as that collected from the vena cava.

The results of these experiments are given in Table IV, in the sixth, seventh and eighth columns of which the figures standing opposite D represent the percentages of sugar in the blood of the portal vein, and those opposite V, the percentages in that of the vena cava. That is to say, the figures occupying the position of numerators represent the values of the portal blood; the denominators, those of the blood of the vena cava. The numbers are arranged in each column in the order in which the samples of blood were withdrawn from either vein.

It will be seen that, almost without exception, the percentage of sugar in the inflowing and that in the outflowing blood of the liver are equal before any injection of sugar is made (see previous paper). In column 7 the change produced by the injection of sugar is recorded.

NO.	WT.	FOOD	GLYCOGEN IN LIVER	DEXTROSE INJECTED IN 5 MIN.	6 PER CENT DEXTROSE IN BLOOD SAMPLES: Before injection	7 PER CENT DEXTROSE IN BLOOD SAMPLES: During injection	8 PER CENT DEXTROSE IN BLOOD SAMPLES: Following injection	9 INTERVAL BETWEEN SAMPLES FROM SAME VEIN	10 INTERVAL BETWEEN SAMPLES FROM DIFFERENT VEINS
	kg.		*per cent*	*gr.*					
63	17.4	Starved	Trace	0.84	D $\frac{0.13}{C}$ *0.13', $\frac{0.13}{0.13}$', $\frac{0.13}{0.13}$	$\frac{0.143}{0.143}$', $\frac{0.163}{0.163}$', $\frac{0.178}{0.178}$	$\frac{0.178}{0.178}$', $\frac{0.143}{0.163}$', $\frac{0.143}{0.162}$	1 min.	1 min.
65	5.2	Starved	Trace	0.40	D $\frac{0.170}{V}$ $\frac{0.168}{0.168}$', $\frac{0.168}{0.167}$'	$\frac{0.199}{0.167}$', $\frac{0.248}{0.235}$', $\frac{.....}{0.241}$	$\frac{0.212}{0.212}$', $\frac{0.199}{0.226}$', $\frac{0.185}{0.199}$	1 min. but 2-3 min. for last 3	1 min.
70	22.3	Starved	0.337	0.48	D $\frac{0.133}{V}$ $\frac{0.133}{.....}$', $\frac{0.133}{0.146}$	$\frac{.....}{0.134}$', $\frac{0.141}{0.146}$', $\frac{0.159}{0.158}$	$\frac{0.150}{0.154}$', $\frac{0.154}{0.166}$	1 min. but 2-3 min. for last 3	1 min. but 0.5 min. for last 3
70a	22.3	Starved	0.337	0.48	D $\frac{0.166}{V}$ $\frac{0.166}{0.166}$	$\frac{0.169}{0.166}$', $\frac{0.166}{.....}$	$\frac{0.182}{0.179}$', $\frac{0.182}{0.179}$', $\frac{0.182}{.....}$	2-3 min.	0-5 min.
68	14.3	Moderately fed	0.675	0.64	D $\frac{0.100}{V}$ $\frac{0.100}{0.100}$', $\frac{0.099}{0.098}$', $\frac{0.100}{0.100}$	$\frac{.....}{0.111}$', $\frac{0.106}{0.110}$', $\frac{0.111}{0.111}$', $\frac{0.118}{0.118}$	$\frac{0.113}{0.103}$', $\frac{0.108}{0.108}$', $\frac{0.108}{0.107}$	1 min.	1 min.
68a	14.3	Moderately fed	0.675	0.52	D $\frac{0.108}{V}$ $\frac{0.108}{0.110}$', $\frac{0.111}{.....}$	$\frac{.....}{0.114}$', $\frac{0.118}{0.118}$	$\frac{0.116}{0.119}$	1-2 min.	1-2 min.
71	6.8	Well fed?	Trace	0.52	D $\frac{0.137}{V}$ $\frac{0.137}{0.140}$', $\frac{0.136}{0.140}$', $\frac{0.126}{0.140}$	$\frac{0.181}{0.143}$', $\frac{(0.236)}{0.177}$	$\frac{0.148}{0.181}$', $\frac{0.143}{0.170}$', $\frac{0.154}{.....}$	2 min.	0.5 min.
71a	6.8	Well fed?	Trace	0.48	D $\frac{0.194}{V}$ $\frac{0.194}{0.184}$'	$\frac{0.215}{(0.143)}$', $\frac{0.215}{0.220}$	$\frac{0.184}{0.196}$', $\frac{0.140}{0.184}$	2 min.	0.5 min.
72	12.6	Well fed	6.25	0.48	D $\frac{0.200}{V}$ $\frac{0.200}{0.200}$', $\frac{(0.244)}{0.207}$	$\frac{0.220}{0.217}$', $\frac{0.220}{0.215}$', $\frac{0.220}{0.222}$	$\frac{.....}{0.200}$', $\frac{0\ 220}{0.220}$	2 min.	0.5 min.
72a	12.6	Well fed	6.25	0.52	D $\frac{0.220}{V}$ $\frac{0.220}{0.249}$', $\frac{0.220}{0.243}$	$\frac{0.249}{0.200}$', $\frac{0.268}{0.250}$', $\frac{0.333}{0.377}$	$\frac{0.350}{0.392}$', $\frac{.....}{.....}$', $\frac{0.332}{0.332}$	2 min. before injection; 1 2 during	0.5 min.
74	8.7	Well fed	4.12	0.52	D $\frac{0.160}{V}$ $\frac{0\ 160}{0.160}$', $\frac{0.148}{0.160}$', $\frac{0.160}{0.160}$	$\frac{0.190}{0.160}$', $\frac{0.181}{0.192}$	$\frac{0.167}{0.183}$', $\frac{0.182}{0.186}$', $\frac{0.172}{0.188}$	2 min.	0.5 min.
74a	8.7	Well fed	4.12	0.40	D $\frac{0.176}{V}$ $\frac{0.176}{0.176}$'	$\frac{.....}{0.186}$', $\frac{0.193}{0.197}$	$\frac{0.191}{0.197}$', $\frac{0.176}{0.188}$', $\frac{0.148}{0.188}$	2 min.	0.5 min.
75	5	Well fed	4.88	0.36	D $\frac{0.158}{V}$ $\frac{0.158}{0.166}$', $\frac{0.138}{0.158}$', $\frac{.....}{0.158}$	$\frac{0.207}{0.187}$ (stopped breathing; resuscitation)		2 min.	0.5 min.
75a	5	Well fed	4.88	0.36	D $\frac{0.204}{V}$ $\frac{0.204}{0..267}$', $\frac{0.204}{0..330}$	$\frac{0.345}{0.345}$', $\frac{.....}{0.395}$	$\frac{0.345}{0.475}$', $\frac{0.332}{0.489}$', $\frac{0.332}{0.496}$ *	2 min.	0.5 min.

*Carotid artery.
*Blood pressure 40 mm.

In the two-minute interval that was allowed to elapse between the beginning of the sugar injection and the collection of the next sample of blood, a detectable increase in sugar concentration had occurred in the portal blood (i.e., numerators) in nearly every instance. In the case of the cava blood (i.e., denominators), however, the increase in sugar concentration in the two-minute interval was not nearly so frequent (e.g., it was absent in Nos. 65, 70, 70 a, 71, 71 a, 72 a and 74). This delay in the appearance of the injected sugar in the 'cava' blood is very evident in the curves which will be referred to immediately. It seems to occur as frequently in well-fed as in starved animals.

The delay is no doubt partly due to the time occupied by the blood in passing through the liver vessels, but this can not account for all of it, as the following rough calculations will show. In a dog weighing 12 kg., which is about the average of those employed in the experiments, and the liver weight 400 grams, there would be from 1300–1500 cc. of blood passing the organ in five minutes, say 550 cc. in two minutes. Assuming that the liver contains 30 per cent of the total blood in the body (i.e., $\frac{30 \times 800}{100} = 240$ cc.), it will be seen that the blood in the organ before the injection started would have been displaced at least twice during the first two minutes of the injection. Part of the delay must, therefore, depend on diffusion of the excess of dextrose into the lymph and tissue juices of the liver, a diffusion which presumably goes on until blood and tissue juices contain the same concentration of dextrose.

When we compare the sugar concentrations in the samples of blood removed from the two vessels during the last three minutes of the injection periods, they are seen to be practically identical in all of the experiments, except 71 and 72 a. From the neighboring values we are inclined to attribute the result in No. 71 to experimental error. That in No. 72a is undoubtedly due to the progressive hyperglycogenolysis present in this case.

The figures in column 8 furnish us with information regarding the after-effects of the sugar injection, the first samples being usually taken two minutes after discontinuing the injection, and the others at subsequent two-minute intervals. Taking the change in the cava-blood first, it will be seen that in twelve of the experiments (Nos. 63, 65, 70, 70 a, 68, 68 a, 71, 72, 72 a, 74, 74 a, 75) there was no return to the normal level within four or five minutes after discontinuing the injection, such a return being evident only in one experiment (No. 71 a).

Similar comparison of the portal blood reveals a more rapid return

to the normal sugar level in five or six of the experiments (Nos. 63, 65, 70?, 71, 71 a, 74 a), but in the remainder the increase in sugar concentration remained as high as in the cava-blood. These results indicate that there can have been no retention of sugar by the muscles of the body within the time of the observation, so that the blood entering the portal radicles in the intestine contained a concentration of dextrose which was almost equal to that leaving the liver. The dilution due to admixture of this blood with blood from the upper extremities and head was not sufficient to make an appreciable difference. On the other hand, in the experiments in which the sugar level in the portal blood fell more rapidly than that of the cava-blood, it must be concluded that muscle retention of dextrose had occurred. We hope to publish further observations on this question in a subsequent communication. It is of interest to compare this result with that noticed when the increase of sugar in the cava-blood is entirely due to hepatic hyperglycogenolysis (see previous paper).

Returning to our main question, as to whether any difference exists between glycogen-poor and glycogen-rich livers in their avidity for dextrose, we may say that there is no evidence, as judged from the difference between the sugar concentrations of portal and cava-blood, of any difference.

In order to give a clearer presentation of the results than is possible in a table, the curves in figure 1 have been plotted from three of the experiments (LXX, LXXI and LXXIV). The dotted line represents the percentage of sugar in the portal blood, and the discontinuous line, that in the portal vein. The following points should be noted:

1. The quicker rise in sugar concentration in the portal blood than in that of the vena cava.

2. The delay, after discontinuing the injection, in the return to the normal level of the sugar concentration of the blood of the vena cava and frequently also in that of the portal vein.

CONCLUSIONS

The existing glycogen-content of the liver does not demonstrably influence the rate with which this organ removes dextrose from the blood of the portal vein.

The authors wish to acknowledge the valuable assistance of Messrs. M. and H. Mahrer in the preparation of this paper.

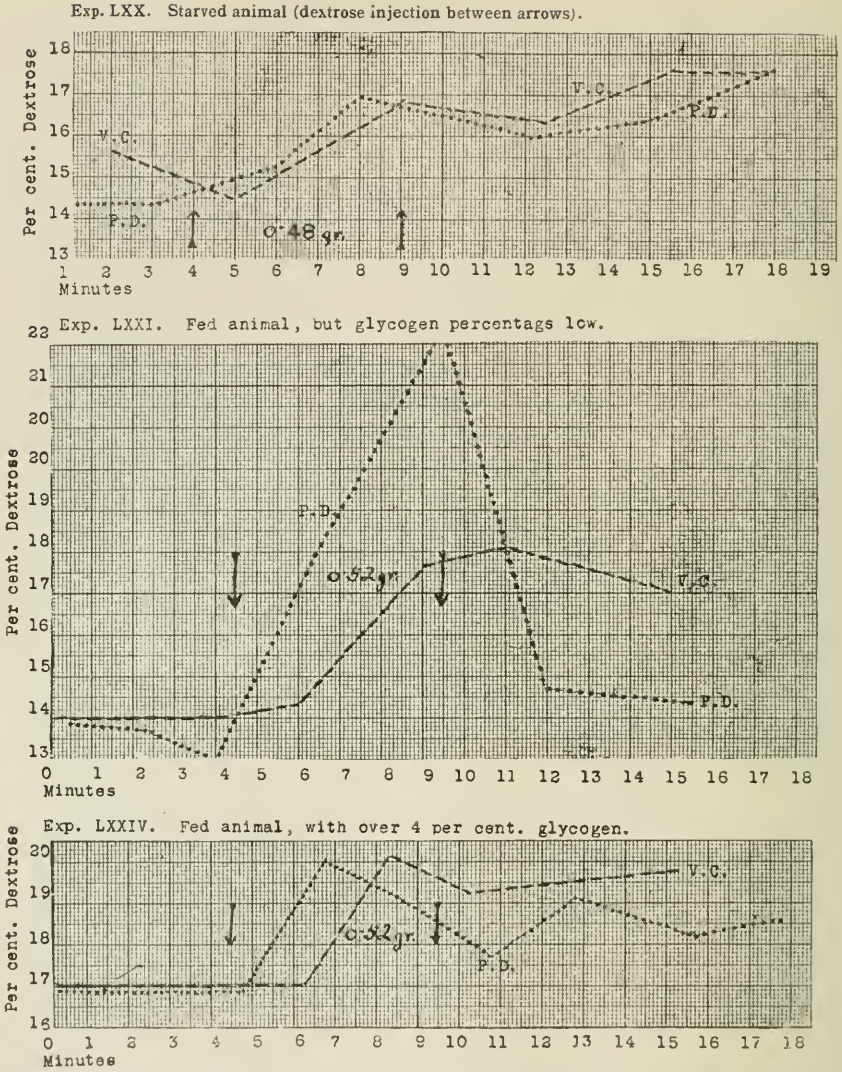

Fig. 1. Curves showing effect of dextrose injections into the portal vein on the sugar content of the blood of the portal vein and vena cava.

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(1) Bang: Der Blutzucker, Wiesbaden, 1913; Jacobson: Biochem. Zeitsch., 1913, lvi, 471; Boë: ibid, 1913, lviii, 106; Wel.: Arch. f. exper. Path. und Phar., 1913, lxxiii, 159.
(2) Macleod and Pearce: This Journal, 1914, xxxv, 87.

THE DIFFERENTIAL EFFECTS OF ADRENIN ON SPLANCHNIC AND PERIPHERAL ARTERIES

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The opposed action on arterial blood pressure, in the dog and cat, of different amounts of adrenin injected intravenously—a rise, or a rise and fall, after large doses; and a pure fall after small doses—present phenomena of considerable theoretical interest. This is especially true in the relation of adrenin to the sympathetic system, and in view of present rather meager evidence of vasodilator influences effected through that system. In studies previously made in this laboratory[1] it was proved that the same amount of adrenin might have opposed effects under different circumstances. Thus the depressor influence of a given small dose when normal arterial pressure prevails is changed to a pressor influence if the arterial pressure has been carried below a certain level, as, for example, by pithing. These opposed effects indicate that adrenin may operate on antagonistic processes, and, as an alternative to the idea of dilator and constrictor sympathetic actions, the tentative suggestion was offered that adrenin causes relaxation of blood vessels when they are tonically contracted, and contraction when they are relaxed. Another natural opposition in the vascular system which has been claimed by various observers[2] is one existing between the vessels of the splanchnic area and those of the periphery. The present investigation was directed towards an answer to the question whether adrenin has differential effects on these two vascular regions.

The cats used in these experiments were anesthetized with urethane (2.0 g. per kilo, by stomach). The form of adrenin employed was fresh adrenalin diluted with distilled water just before each experiment. Unless otherwise stated the concentration was 1: 100,000. Injections were made into the jugular vein from a syringe graduated to fiftieths of a cubic centimeter. In most cases injections of 0.2 cc. were made, at a

[1] Cannon and Lyman: This Journal, 1913, xxxi, 385.

[2] Dastre and Morat: Système Nerveux Vaso-moteur, Paris, 1884, 329.

uniform rate in each instance, over a period of from fifteen to thirty seconds.

Blood pressure was registered by means of a mercury manometer, connected usually with one of the carotid arteries. In a few cases the pressure changes in the nasal mucosa were recorded by a membrane manometer connected with one of the anterior nares, the other being plugged with vaselined cotton and the posterior nares being closed with Mendenhall's apparatus.[3]

The vasodilator effect of small doses of dilute adrenin is, in our experience, a constant phenomenon rather than "a somewhat variable effect," as Dale[4] describes it. Of the fifty-three animals used in this series of experiments only five failed to show a fall of blood pressure when injected with 0.2 cc. adrenalin, 1:100,000. As will be shown later, four of these were in an abnormal condition which would account for the failure. Of the forty-nine normal cats, therefore, in only one did the small standard dose of adrenalin fail to cause a drop of arterial pressure.

The response of peripheral arteries. In order to determine the response of the peripheral arteries to this small dose of adrenalin, the circulation was excluded from the splanchnic region by tying the inferior and superior mesenteric arteries and the coeliac axis, and sometimes also the renal arteries. Of twenty-three animals with splanchnic vessels thus tied, fourteen still showed the characteristic fall of arterial pressure after injection of the standard dose. In four animals in which there had been a preliminary rise of pressure followed by a fall the same dose failed to cause the rise after the splanchnic vessels had been excluded. In two, showing only a rise previous to the tying, the adrenalin produced only a fall after the tying. And in another case a fall of 20 mm. (14 per cent) before splanchnic exclusion was increased to 38 mm. (32 per cent[5]) afterwards.

These results clearly indicate that a small dose of adrenalin (0.2 cc., 1:100,000) causes relaxation of the peripheral arteries. In no case did constriction result. That in some instances the fall was not increased after splanchnic exclusion was probably due to lessened tone in the peripheral arteries as a result of the operation. With such lessened tone the adrenalin would probably not have so great an influence as in conditions of greater tone. This explanation is supported by the obser-

[3] Mendenhall: This Journal, 1914, xxxvi, 59.

[4] Dale: Journ. Physiol., 1913, xlvi, 291.

[5] The blood pressure which had been 143 mm. before tying the splanchnic vessels was lowered to 118 mm. by the operation.

vation that immediately after the operation the standard dose of adrenalin evoked no response, but as time elapsed it brought forth the characteristic dilation.

The response of splanchnic arteries. To find the response of splanchnic arteries the abdominal aorta above the iliacs, both subclavians and both carotids were tied. There remained for the circulation the vessels supplying the trunk and the thoracic viscera as well as those of the splanch-

TABLE I

Effect of excluding the limb and neck arteries upon the response to 0.2 cc. adrenalin, 1:100,000. Pressures are in mm. of mercury. The same dose of adrenalin was given in each case.

BEFORE TYING				AFTER TYING PERIPHERAL ARTERIES		
Cat	Pressure	Adrenalin caused a fall of	Per cent fall	Pressure	Adrenalin caused a rise of	Per cent rise
	mm.	*mm.*		*mm.*	*mm.*	
I	104	23	22.1	150	2	1.3
				72	7	9.7
II	154	21	13.6	157	11(fall)	7.0 (fall)
				104	6(rise)	5.7 (rise)
III	135	9	6.7	131	10	7.6
IV	115	30	26.0	81	12	14.8
V	113	17	15.0	99	11	11.1
VI	110	20	18.1	55	14	25.4
VII	124	28	22.5	75	30	40.0
VIII	112	32	28.5	131	8	6.1
				80	26	23.5
IX	109	12	11.0	140	12	8.5
X	108	16	14.8	44	17	38.6
XI	112	24	21.4	60	8	13.3
XII	106	20	18.8	108	15	13.8
XIII	94	32	34.0	107	23	21.4
XIV	132	36	27.2	95	4	4.2
XV	138	18	13.0	140	16	11.4
XVI	90	26	28.8	48	7	14.5

nic region. Adrenin is without effect on the pulmonary arteries;[6] it typically causes relaxation of the coronaries,[7] but since the coronary vessels are not capacious they need not be considered. So far as the blood supply to the trunk is concerned, it is probably of much smaller

[6] Brodie and Dixon: Journ. Physiol., 1904, xxx, 488.

[7] Langendorff: Zentralbl. f. Physiol., 1907, xxi, 555; Cow: Journ. Physiol., 1911, xlii, 132.

volume than that of the large and extensive abdominal organs.[8] It is justifiable, therefore, to regard the animal operated upon as described above as carrying on mainly a splanchnic circulation, and to consider the influence of adrenin as exerted chiefly upon the splanchnic vessels. The limb and neck arteries were tied in twenty animals. The operation invariably resulted in a marked increase in arterial pressure.

In all but two of the twenty cases the standard dose of adrenalin (0.2 cc., 1: 100,000), injected after the limb and neck arteries were tied,

TABLE II

Effect of excluding alternately the peripheral and splanchnic arteries upon the response to 0.2 cc. adrenalin, 1:100,000. Blood pressure is expressed in millimeters of mercury. The Roman numerals show the order of clamping and unclamping the arteries.

	NORMAL			PERIPHERAL ARTERIES CLAMPED			UNCLAMPED			SPLANCHNIC ARTERIES CLAMPED		
Cat	Pressure	Adrenalin caused fall of	Per cent fall	Pressure	Adrenalin caused rise of	Per cent rise	Pressure	Adrenalin caused	Per cent	Pressure	Adrenalin caused fall of	Per cent fall
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*	
				I			II			III		
A	124	27	21.7	73	32	43.8	83	19 fall	22.8 fall	110	10	9.1
				III			II			I		
B	111	17	15.3	73	5	6.8	68	5 rise	7.3 rise	No effect		
				III			II			I		
C	112	32	28.5	132	8	6.0	118	17 fall	14.4 fall	128	15	11.8
				80	26	32.5						
				I			II			III		
D	135	18	13.3	132	9	6.8				113	36	31.8

caused a rise of arterial pressure—an effect just the opposite of that caused by the same dose before the tying. Often when the pressure had been greatly increased by the tying, the standard dose at first produced a fall of pressure, but later, as the pressure lessened, the fall was changed to a rise. Moreover, as the pressure decreased below normal the percentage rise after the standard dose usually increased.

In Table I the figures show the change from a fall to a rise when the peripheral arteries are tied.

[8] Ranke: Die Blutvertheilung, Leipzig, 1871, 69.

Splanchnic and peripheral effects in the same animal. In four animals both splanchnic and peripheral responses were obtained after adrenalin injection by clamping first one group of arteries and then the other after the first had been released. The splanchnic arteries were clamped first in two cases, and the peripheral arteries were clamped first in the other two. The results are shown in Table II. In each case the dose of adrenalin was 0.2 cc., 1:100,000.

In every case clamping of the peripheral arteries, whether primary or secondary to splanchnic exclusion, caused the vascular response to the standard dose of adrenalin to change from the normal relaxation (fall) to contraction (rise) (see fig. 1). And in every case except one (B),

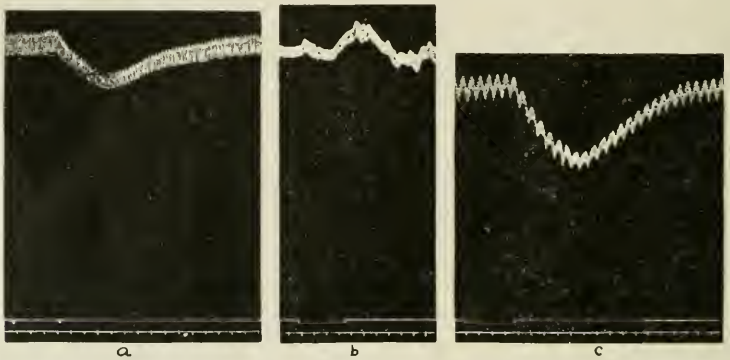

Fig. 1. Effect of injecting 0.2 cc. adrenalin, 1:100,000: a. Upon the whole circulation; b. Upon the arteries of the splanchnic area (peripherals clamped); c. Upon the arteries of the limbs (splanchnic arteries clamped). Time interval five seconds. Middle line, pressure base line (0 mm).

either primary or secondary clamping of the splanchnic arteries continued the normal drop in pressure resulting from the standard dose of adrenalin, though the drop in some instances was not so great as before. In D, however, the fall of 13 per cent under normal conditions was increased to 31 per cent after the splanchnics were clamped. The failure of effect in B may have been due to a too-great relaxation of the vessels as a result of the operation In A and C after the release of the peripheral and the splanchnic vessels adrenalin produced the usual fall. The rise in B may be explained as a splanchnic effect, in the presence of relaxed peripheral vessels (note the low pressure, 68 mm.).

Cases in which dilute adrenalin caused a rise in blood pressure. Occasionally there are animals in which no fall in arterial pressure results

from doses of adrenin which normally cause a fall, nor can a fall be obtained by diminishing the dose. As already stated, five cases out of fifty-three were found in which no fall occurred after the standard dose. It will be recalled that all but one of these animals were in poor physical condition. One animal had been suffering from urethritis for about three weeks and had eaten insufficiently during that time. It had decreased in weight from 2.9 to 2.4 kilos. The blood pressure was 58 mm. of mercury; 0.2 cc. adrenalin, 1: 100,000, injected at the same rate as in other experiments resulted in 17–18 mm. rise in blood pressure. Even 0.2 cc. adrenalin 1: 1,500,000, caused in some tests a slight rise. In no test was the slightest fall in pressure obtained.

In a second case the animal was pregnant and appeared poorly nourished, for its ribs were very prominent. The blood pressure was about 95 mm. of mercury, and nothing but a rise could be induced, even with as little as 0.02 cc. adrenalin, 1:100,000. The arteries of the splanchnic area were tied, whereupon five or six times as much adrenalin was required to cause a rise equivalent to that obtained before the occlusion of the splanchnic arteries (sufficient time was allowed to elapse for the recovery of vasomotor tone). Apparently the primary rise had been due largely to contraction of the splanchnic area and the remainder of the arterial system was not responsive, in the direction of contraction, to the standard minute dose.

Fig. 2. Effect of 0.2 cc. adrenalin, 1:100,000 upon a cat that was ill: a. Upon whole circulation; b. Upon peripheral arteries (splanchnics clamped). Nasal plethysmograph line just above pressure base line (0 mm). Time line at bottom, interval half minutes. A fall in plethysmograph curve indicates constriction.

A third animal, whose blood pressure was but 67 mm. of mercury, was diseased; in this animal 0.2 cc. adrenalin, 1: 100,000, caused a rise of 7 mm. (fig. 2), but after the splanchnic arteries were tied the same dose caused a fall of only 3 to 6 mm. This experiment, therefore, indicates that the rise, with very dilute adrenin, in animals in weakened condition, is due to constriction of the splanchnic arteries and also to lessened dilation of the peripheral vessels.

A fourth animal which had been given alcohol, failed to show a definite fall with dilute adrenalin.

No explanation was to be had for the failure of the depressor effect in the fifth animal.

In three of the five, however, there was clear evidence of a low blood pressure, a condition that harmonized with the atonic state of the animals. The indication that toneless peripheral vessels are not made to relax further by injections of adrenin has already been mentioned. It seems reasonable to assume, therefore, that when animals are in poor physical condition, a small dose of adrenin fails to cause relaxation of the vessels that normally relax for such a dose, because the tone of these vessels is already low.

Depressor action of adrenin as affected by low arterial pressure. If low arterial pressure was the occasion for failure of adrenin to produce a fall of arterial pressure in the foregoing cases, it becomes a matter of interest to learn whether an induced low pressure will affect the action

TABLE III

Effect of lowering the arterial pressure, by hemorrhage, and then raising it, by restoring the lost blood, upon the response to the standard dose of adrenalin

BLOOD PRESSURE IN MM. OF MERCURY	CHANGE IN PRESSURE IN RESPONSE TO 0.2 CC. ADRENALIN 1: 100,000	PER CENT CHANGE
mm.	*mm.*	
102	14 fall	13.7 fall
83 from hemorrhage	6 fall	7.2 fall
71 from hemorrhage	5 rise	7.0 rise
65 from hemorrhage	8 rise	12.3 rise
77 from injection	4 rise	5.1 rise
87 from injection	4 rise	4.6 rise
94 from injection	4 rise 2 fall	2.1 fall
99 from injection	5 rise 5 fall	5.0 fall
109 from injection	2 rise 12 fall	11.0 fall

of the substance. Low pressure was established in two different ways, by hemorrhage, and by depressor stimulation.

(*a*) *Low pressure due to hemorrhage.* In seven experiments there was one in which the primary fall of arterial pressure due to injected adrenalin was changed to a rise as the pressure was reduced by hemorrhage from 102 mm. to 65 mm. The drawn blood was defibrinated and returned to the circulation by degrees. As the pressure rose in consequence of the restored blood the depressor action of adrenalin gradually reappeared.

Table III shows in this case the change in response to 0.2 cc. adrenalin, 1: 100,000, as the pressure was lowered and then raised again by injection of defibrinated blood.

In the remaining six experiments there was merely a smaller fall, following the standard dose of adrenalin, as the blood pressure was lowered by bleeding, until at about 40–50 mm. there was either no effect or a slight rise and fall. That no considerable rise occurred may have been due to a temporary abolition of the splanchnic response as a result of hemorrhage, for very little time elapsed between the bleeding and the injection.

The effect of the standard dose of adrenalin was next studied in animals with splanchnic circulation excluded and with blood pressure lowered by hemorrhage. In the five cases studied nothing but a fall in pressure occurred even when the pressure had been lowered to 40 or 50 mm. (fig. 3). At 20 to 30 mm. the dose had no effect. The figures in

TABLE IV

Response to the standard dose of adrenalin in an animal with the splanchnic arteries tied, when the pressure is lowered by hemorrhage

BLOOD PRESSURE IN MM. OF MERCURY	RESPONSE TO 0.2 CC. ADRENALIN 1:100,000	PER CENT FALL
mm.	*mm.*	
161	12 fall	7.4
135	12 fall	8.8
129	18 fall	13.9
110	13 fall	11.8
97	15 fall	15.4
84	13 fall	15.4
71	18 fall	25.3
50	10 fall	20.0
34	6 fall	17.6

Table IV present a good example of the response to adrenalin (0.2 cc., 1:100,000) when in an animal with splanchnic circulation excluded, the blood pressure is decreased by hemorrhage.

Judging from the experiments described earlier in this paper one would expect, in an animal with the peripheral arteries tied, that the rise in pressure, in response to adrenin would continue as the pressure was lowered by bleeding. This was found to be the case in the only animal in which it was tried. The rise with 0.2 cc. adrenalin persisted with the pressure as low as 10 mm. (fig. 4). At 70 mm. pressure the dose caused 8 mm. or 11.4 per cent rise. At 29 mm. the same dose caused 8 mm. or 27.5 per cent rise. At 10 mm. the same dose caused 3 mm. or 30 per cent rise.

Fig. 3.

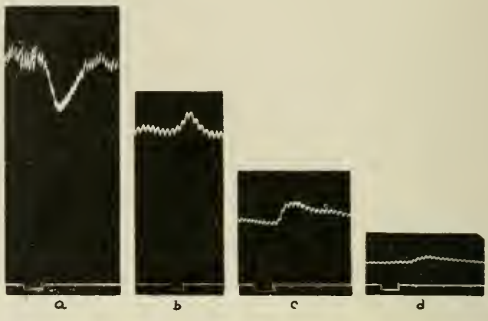

Fig. 4.

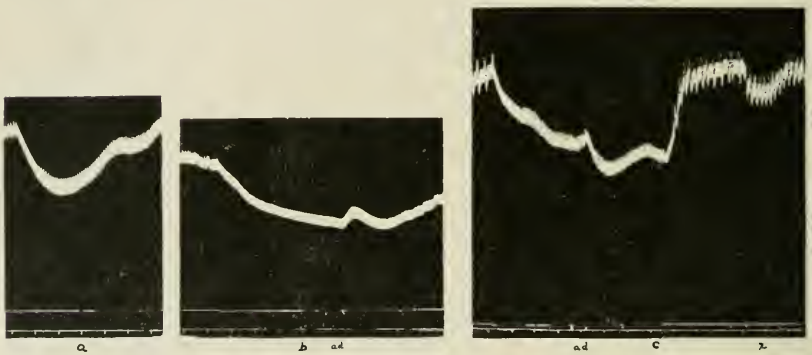

Fig. 5.

Fig. 3. Effect of 0.2 cc. adrenalin, 1: 100,000 after hemorrhage, with arteries of splanchnic area tied: a. Before bleeding; b. 65 cc. of blood removed; c. 35 cc. additional blood removed. Time half minutes. Lower line pressure base line (0 mm).

Fig. 4. Effect of 0.2 cc. adrenalin, 1: 100,000 after hemorrhage with peripheral arteries tied: a. Before tying peripheral arteries; b. After tying peripheral arteries; c. 35 cc. of blood removed; d. 28 cc. additional blood removed. Time interval half minutes. Base line zero pressure.

Fig. 5. Effect of 0.2 cc. adrenalin, 1: 100,000 when blood pressure is lowered by stimulation of the depressor nerve: a. Without depressor stimulation; b. Pressure lowered by depressor stimulation, showing rise with adrenalin; c. Pressure lowered by depressor stimulation, showing fall with adrenalin (different animal). Adrenalin injected at *ad*. *x* shows effect of same dose of adrenalin with normal pressure. Time half minutes.

(*b*) *Low pressure due to depressor stimulation.* When blood pressure is lowered by stimulation of the depressor nerve, 0.2 cc., adrenalin, 1:100,000, may cause, as shown by Cannon and Lyman,[9] a fall, a rise and fall or a pure rise depending to a certain extent upon the height of the pressure when injected (fig 5). In cases where a rise occurs, it disappears if the splanchnics are tied. Seven animals were used in

TABLE V

Effect of stimulation of the depressor nerve upon the response to the standard dose of adrenalin (0.2 cc., 1:100,000). Pressures are expressed in mm. of mercury.

NORMAL				AFTER DEPRESSOR STIMULATION				
							Splanchnics tied	
Animal	Pressure	Effect of adrenalin	Per cent change	Pressure from depressor stimulation	Effect of adrenalin	Per cent change	Pressure from depressor stimulation	Effect of adrenalin
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	
A	108	3 rise 13 fall	12.0	65	9 rise	13.8	74	No effect
B	115	30 fall	26.0	85	14 rise	16.4	114 (158 mm. after tying splanchnics)	14 fall
C	122	10 fall	8.1	67	19 rise	28.3	80	No effect
D	120	19 fall	15.8	93	15 rise 7 fall	5.3 7.5		
E	89	28 fall	31.4	43	5 rise	11.6		
F	126	12 fall	9.5	90	13 fall	14.4		
G	102	15 fall	14.7	90 73 49	2 rise 13 fall 4 rise 4 fall 3 rise	2.2 14.4 5.4 5.4 6.1	42	No effect

studying the effect of dilute adrenalin when pressure was lowered by depressor stimulation. In every case both vagi were cut. Standard doses of adrenalin (0.2 cc., 1:100,000) were invariably injected. Table V shows the results of these experiments.

As the results in Table V indicate, exclusion of the splanchnic area causes adrenin to have no pressor effect when the general blood pressure

[9] Cannon and Lyman: Loc cit., 387.

has been lowered by depressor stimulation to a point where the standard dose would naturally produce a rise.

In the foregoing experiments the evidence obtained by simple tying out of the splanchnic region or of the neck and limb vessels has been supported. If arterial pressure has been lowered either by hemorrhage or by depressor stimulation the standard dose of adrenin, instead of producing a fall of pressure, produces a rise or has no effect. If now the splanchnic area is excluded adrenin fails to have any pressor influence and may indeed drop the pressure still further. If, on the contrary, the peripheral arteries are tied, the same dose causes an elevation of the lowered pressure, even when 10 mm. measures the arterial tension.

Latent period and duration of the adrenin effects. Since the splanchnic and peripheral arteries respond in opposite directions to minute doses of adrenin, and yet the end result, when both portions of the circulation are affected, is the characteristic fall of pressure which is seen when the peripheral vessels alone are involved, the time relations of the response in the two vascular regions becomes a question of considerable interest. Tests were made on eight animals, and by records written on a rapidly moving drum the latent period and the duration of the adrenin effect were carefully estimated. The results are presented in Table VI.

In the cases of a pure drop in pressure the latent period varied from 12.5 to 22 seconds, with an average of 15.7 seconds. The average latent period for the splanchnic rise (after tying the peripheral arteries) was 18.6 seconds. It seemed probable, therefore that with a minute dose of adrenin, peripheral dilation precedes splanchnic constriction and consequently masks it. There is a possibility, however, that the operation of tying or clamping off part of the circulatory system tends to lengthen the latent period—a suggestion that receives support from an observation that a fall of pressure which occurred in 12.6 seconds before operation did not occur after splanchnic exclusion until 26 seconds had elapsed.

If the foregoing suggestion is correct, the discrepancy between the latent periods of the peripheral relaxation and the splanchnic contraction would not be so great as the figures in Table VI indicate. Indeed in two of the cases there was first a rise of blood pressure and then a fall. With somewhat larger doses of adrenin than those here given this is a usual result. Since the standard dose causes both a splanchnic contraction and a peripheral relaxation of arteries, the common occurrence of a pure fall of pressure, when both these parts of the circulatory system are affected, indicates that this small amount of adrenin usually affects first the peripheral vessels.

The duration of the fall in pressure after the standard dose (0.2 cc. adrenalin, 1: 100,000) averaged in five animals 59 seconds. The average duration of the splanchnic rise in five animals, however, was only 37.8 seconds. In no single case did the splanchnic rise last more than three-fourths as long as the normal fall. With the amount of adrenin used in these experiments, therefore, the dilation of the peripheral arteries begins earlier and lasts longer than the constriction of the splanchnic arteries. The result is that the splanchnic rise is masked by the peripheral fall of pressure.

TABLE VI

Latent period and duration of adrenin effects. In each case 0.2 cc. adrenalin, 1:100,000, was injected. Records were made on a rapidly moving drum

ANIMAL	WEIGHT IN KILOGRAMS	LATENT PERIOD OF THE NORMAL FALL	DURATION OF FALL	LATENT PERIOD OF THE SPLANCHNIC RISE (PERIPHERALS TIED)	DURATION OF RISE
		sec.	*sec.*	*sec.*	*sec.*
A.	2.5	13	70	17	42
B.	2.0	12.6	53	17.5	38
C.	3.2	15	46	18	31
D.	2.5	12.5	66	23	41
E.	2.1	(rise 8)			
		fall 15	(52)	18	37
F.	2.7	20			
G.	3.0	(rise 14)			
		fall 22	(48)		
H.	3.2	16	59		
Average		15.7	59	18.6	37.8

Effect of dilute adrenin on the blood vessels in the nasal mucosa. In view of the opposite effects of dilute adrenin upon the splanchnic and peripheral arteries, the question arose as to whether the dilation of the peripheral vessels extended to the skin or was limited to the more deeply lying vessels. No convenient method was found to determine the action of vessels in the skin areas over the general body surface, but the vascular changes in the nasal mucosa were easily studied. A membrane manometer was connected to one of the nasal openings in the manner described earlier in this paper. The nasal plethysmograph record was made simultaneously with the tracing of general blood pressure.

Doses of adrenalin (0.2 cc., 1: 100,000) which caused dilation of the peripheral arteries always caused constriction of the nasal mucosa (fig. 2). Amounts as small as 0.05 cc. of adrenalin, 1: 100,000, caused constriction. Martin and Mendenhall[10] produced constriction of the nasal mucosa by injecting 0.5 cc. adrenalin, 1: 1,000,000. The reaction of the nasal mucosa to adrenin therefore is of the splanchnic type rather than the peripheral type.

The threshold dose of adrenin changing a fall of blood pressure to a rise. In determining the threshold dose it is necessary to consider the rate[11] of injection as well as the concentration. And since the sensitiveness of tissues to adrenin decreases if the injections are too frequent or too concentrated,[12] care must be exercised to avoid these sources of error.

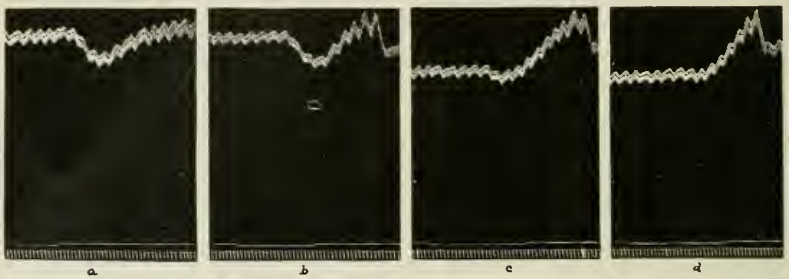

Fig. 6. Threshold of the change from a fall to a rise with adrenalin when splanchnic arteries are tied: a. 0.1 cc. adrenalin, 1: 10,000, fall only; b. 0.25 cc. adrenalin, 1: 10,000, fall and rise; c. 0.3 cc. adrenalin, 1: 10,000, slight fall, large rise; d. 0.35 cc. adrenalin, 1: 10,000, rise only. Time seconds.

The rate of injection was kept nearly constant, being uniform and over a period of from ten to twenty seconds, For convenience two concentrations of adrenalin solution were kept ready, viz., 1: 100,000 and 1: 10,000. To prevent dilution in the process of changing the solutions one solution was injected into the external jugular vein while the other was injected into the femoral vein. In order to avoid the lowering of the sensitiveness of the blood vessels to adrenalin the smallest possible number of doses were injected.

As the evidence already presented has shown that adrenin causes constriction of the arteries of the splanchnic area and relaxation of those of the periphery, the question of the threshold dose of adrenin, changing

[10] Unpublished work done in this laboratory.

[11] Cannon and Lyman: Loc cit., 382.

[12] Elliott: Journ. Physiol., 1905, xxxii, 443.

a fall to a rise, is concerned solely with the effect on the peripheral vessels.

The threshold was determined for three animals, the splanchnic arteries being tied off in each case. The threshold for two of these was between 0.2 cc. and 0.3 cc. of 1: 10,000 adrenalin while in the third it was about 0.1 cc. of 1: 10,000 adrenalin. The third animal weighed but 2.0 kilos while the others weighed 3.7 and 3.4 kilos. As the threshold was just passed a preliminary fall preceded the rise (fig. 6). The threshold of the change of a fall to a rise apparently varies with the individual. The experiments were too few in number to permit definite conclusions but they indicate the magnitude of the threshold.

Fig. 7. Effect of small doses of ergotoxine phosphate in increasing response of splanchnic arteries to adrenalin (peripheral arteries tied): a. Effect of 0.2 cc. adrenalin, 1:100,000, before giving ergotoxine; b. Effect of same dose of adrenalin after giving two doses of ergotoxine 0.5 mgm. and 0.6 mgm., respectively. Time half minutes.

Reversal of the adrenin effect after ergotoxine. Dale's[13] discovery, that after ergotoxine a dose of adrenin that would normally cause an increase of arterial pressure causes a decrease, is of considerable interest in connection with the reversed effects of adrenin on peripheral and splanchnic arteries. Since the alteration induced by ergotoxine is from contraction to relaxation it was necessary to make the test on animals with the peripheral arteries tied. Under these circumstances does ergotoxine cause the usual increase of pressure resulting from the standard dose of adrenin to change to a decrease?

It was impossible to obtain the "reversal effects" after the injection of small doses of ergotoxine phosphate (e.g., 0.5 mgm.). Indeed these small doses seemed to render the splanchnic response more sensitive, for the rise of pressure after adrenalin injection was thus increased (see fig. 7). Reversal or inhibition of the splanchnic constriction was obtained only after several larger doses (5 to 10 mgm.) of ergotoxine, such as Dale used, had been administered (see fig. 8). Thus after such large doses, adrenalin (0.4 cc., 1: 10,000) caused, in an animal with limb and neck arteries tied, a fall of arterial pressure from 86 mm. to 65 mm. Recovery required six minutes. When 1 cc. of this solution was injected there was a similar fall, which was recovered from after eleven minutes.

[13] Dale: Journ. Physiol., 1905, xxxii, lix.

Discussion of the opposed action of splanchnic and peripheral arteries in response to dilute adrenin. Cannon and Lyman[14] proposed the idea that adrenin causes relaxation of the blood vessels when they are tonically shortened,—contraction when they are relaxed. The evidence in this research indicates that the opposite action of dilute adrenin depends rather upon opposite effects produced in the splanchnic and peripheral arteries. The rise of pressure when the vessels are relaxed would thus be explicable on the ground that the standard dose of adrenin cannot relax further the peripheral vessels already relaxed but can still have its constrictor influence on the splanchnic area. In certain conditions, however, the state of tonicity may play a part, e.g., when the blood

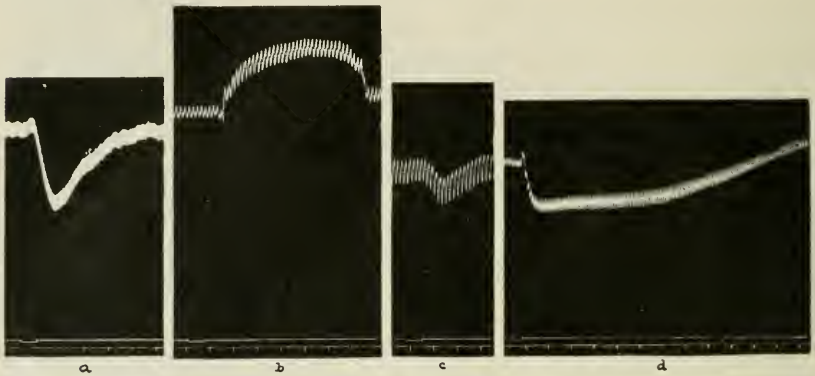

Fig. 8. Reversal of splanchnic arterial response to adrenalin after giving large doses of ergotoxine phosphate: a. Effect of 0.2 cc. adrenalin 1:100,000 before tying arteries; b. Effect of 0.2 cc. adrenalin 1:100,000 after tying peripheral arteries; c. Effect of 0.2 cc. adrenalin 1:100,000 after 21.5 mgm. ergotoxine had been given. d. Effect of 0.4 cc. adrenalin, 1:1,000 after a total of 27.5 mgm. ergotoxine had been given.

pressure has been rendered unusually high by clamping the peripheral arteries, for dilute adrenin then often causes dilation. But even in this case, the dilation might be due to the arteries supplying the trunk, as it is impossible to tie them off.

Although there is no convincing proof that there are vasodilator nerves in the sympathetic system, their presence in the peripheral blood vessels and their absence from, or relatively slight development in, those of the splanchnic area, would offer the most plausible explanation of the differential effects of dilute adrenin. An alternative explanation would

[14] Cannon and Lyman: Loc cit., 398.

be that the arterial muscle differed in the two regions and that adrenin produced its effect by direct stimulation of the plain muscle. The first seems impossible, while the second is contradicted by evidence of Dixon and Brodie[15] that adrenin produces its effect through the sympathetic nerve ending.

If the "sympathetic vasodilator" hypothesis is accepted it is necessary to assume that the vasodilators are more sensitive to adrenin than are the vasoconstrictors, in order to account for the change from a fall to a rise in the response of the peripheral arteries to increasing doses of the drug. The rise which occurs as the vasoconstrictor threshold is passed is preceded by a fall (see fig. 6). This would be expected from adrenin slowly injected because the first amounts reaching the nerve endings might be insufficient to stimulate the less sensitive constrictor endings yet strong enough to affect the vasodilator endings and then as the amount of adrenin increased the vasoconstrictors would be brought into action and overwhelm the vasodilator effect.

The idea that vasodilator and vasoconstrictor nerves can be brought into action in turn by different strengths of stimulation receives support from the observation that weak sensory stimulation may cause a lowering[16] of blood pressure while strong sensory stimulation (20 to 200 times stronger than the stimulation which produces a depressor effect) usually produces a rise.[17] Bowditch and Warren[18] found that a slow rate of stimulation with an induced current caused vasodilation while a more rapid rate caused vasoconstriction. Vasodilators may have been stimulated in the former case and vasoconstrictors in the latter case.

Ostroumoff[19] was one of the earliest to believe that the sympathetic contained vasodilator nerves. He was supported in this belief by Puelma and Luchsinger.[20] Dastre and Morat[21] thought that they had evidence of the existence of vasodilators in the sympathetic. Dale[22] is inclined to accept the theory of the existence of an admixture of vasodilators and vasoconstrictors in the sympathetic and, as he points out,

[15] Dixon and Brodie: Loc cit., 494.

[16] Knoll: Sitzungs. a. Akad. d. Wissensch. zu Wien, Math.-Naturwiss. Klasse, 1885, xcii, Abtheilung, 3, 449.

[17] Martin and Lacey: This Journal, 1914, xxxiii, 222.

[18] Bowditch and Warren: Journ. Physiol., 1886, vii, 447.

[19] Ostroumoff: Pflüger's Arch., 1876, xii, 219.

[20] Puelma and Luchsinger: Pflüger's Arch., 1878, xviii, 489.

[21] Dastre and Morat: Loc cit., 247.

[22] Dale: Loc cit., 299.

this would account for the fact that no reversal can be obtained in the rabbit, vasodilators in the sympathetic being theoretically absent.

The utility of the simultaneous peripheral dilation and splanchnic constriction. It may be that the amount of adrenin, which is poured into the blood stream during times of stress or excitement, is at first of the order of that used in the foregoing experiments. Hoskins and McClure[23] estimated that the amount of adrenin secreted as a result of splanchnic stimulation was at first of this order. Elliott[24] found that the sensitiveness of the arrectores pilorum to adrenin varied with the functional use by each animal. In a similar manner it is possible that response of the blood vessels to adrenin is in accordance with their functional use in times of excitement, there being an active dilation of the peripheral arteries and simultaneously a constriction of the arteries of the splanchnic region. Such an arrangement would assure the motor organs an abundant blood supply for their most efficient action.

SUMMARY

1. Dilute adrenalin slowly injected caused a fall in general blood pressure in 48 out of 53 animals tried. Three of the animals in which a fall did not occur, were in poor physical condition and one was just recovering from the effects of alcohol.

2. Dilute adrenalin caused dilation of the peripheral arteries even after extremely low pressures had been produced by hemorrhage. The same dose of adrenalin caused constriction of the splanchnic arteries.

3. When the blood pressure was lowered by depressor stimulation the same dose of adrenalin caused a fall, a rise and fall or a pure rise depending somewhat upon the height of the pressure.

4. The average latent period for the peripheral fall in the blood pressure (from doses of 0.2 cc. adrenalin, 1: 100,000) was 15.7 seconds; the latent period for the splanchnic rise was 18.6 seconds. The duration of the splanchnic rise was 37.8 seconds, while the duration of the peripheral fall was 59 seconds.

5. The threshold for the change of a fall to a rise in the peripheral arteries, when adrenalin was injected (in three animals only) over a period of from ten to twenty seconds, was between 0.1 and 0.3 cc. of a 1: 10,000 solution.

[23] Hoskins and McClure: Arch., Internal Med., 1912, x, 352.

[24] Elliott: Loc cit., 416.

6. Large doses of ergotoxine phosphate inhibit the splanchnic response to dilute adrenalin. The existence of sympathetic vasodilator nerves in the peripheral arteries and their absence in the splanchnic arteries would account for the opposed action of like doses of dilute adrenalin upon the peripheral and splanchnic arteries.

I wish to thank Dr. W. B. Cannon for suggesting this research and for his advice and criticism.

THE OSMOTIC PROPERTIES OF CALCIUM AND MAGNESIUM PHOSPHATE IN RELATION TO THOSE OF LIVING CELLS

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INTRODUCTION

In 1867 Traube suggested that living cells might owe their semi-permeable properties, at least in part, to precipitates of inorganic substances.[1] In recent times this suggestion has been nearly lost sight of and the attention of physiologists has been chiefly centered on the question whether or not the semi-permeable surface of the cell is composed of lipoid.

Most of the work on this question has been directed toward discovering to what substances the cell surface is or is not permeable, it being tacitly assumed that we know or can predict *a priori* the semi-permeable properties of "lipoids." But the little work that has been done with artificial lipoid membranes is far from justifying this assumption.

It has been shown, for instance, by Nathansohn[2] and Ruhland[3] that artificial lipoid membranes, when soaked with water are quite permeable to substances which are soluble in water, whether or not the substances in question are soluble in lipoids. On the other hand, water-free lipoid membranes are impermeable to water as well as to dissolved substances.

It was shown by Pfeffer[4] that semi-permeable membranes can be made of calcium phosphate, and it is well known that both calcium and inorganic phosphates are present in most living cells. Since Pfeffer's time there has accumulated a considerable body of evidence which shows that calcium plays a very important part in the activities of living tissues, and particularly that the permeability of the cell surfaces is affected by its presence or absence.

[1] Traube: Arch. f. Anat. Physiol., und wissensch. Med.; 1867, xxxiv, 146.
[2] Nathansohn: Jahrb. f. wissensch. Bot., 1904, xxxix, 607.
[3] Ruhland: Jahrb. f. wissensch. Bot., 1909, xlvi, 1.
[4] Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 11.

Thus Loeb[5] finds that $CaCl_2$ can protect the fish, Fundulus, against the toxic effects of KCl. He gives reasons for thinking that the calcium protects by forming an insoluble compound with some "organic anion" at the surface of contact between the fish and the solution (Loc. cit., pp. 320 and 321).

Somewhat more direct in their bearing on this question perhaps are certain experiments of Osterhout on plants. This author gives a series of experiments which indicate that the surface of spirogyra filaments is decidedly more permeable to NaCl than to $CaCl_2$, and that the addition of $CaCl_2$ to an NaCl solution may prevent the latter salt from entering.[6] In another series of experiments he shows that the resistance of the leaf cells of the common kelp (laminaria) to the passage of an electric current may be increased by the action of $CaCl_2$, and gives reasons for thinking that the salt acts by decreasing the permeability of the plasma membrane.[7]

Both Loeb and Osterhout find that the effects of the $CaCl_2$ can be to a greater or less extent imitated by substituting for it certain other salts such as $SrCl_2$, $BaCl_2$, $MgCl_2$, and $La_2(NO_3)_6$. The kations of all these salts form insoluble compounds with the phosphates, and it seems, therefore, not impossible that they may all tend to decrease the permeability of the cells in the same way, namely by impregnating their surfaces with layers of insoluble phosphate.

Both calcium and magnesium are known to be present in most living cells. The physiological experiments make it appear that calcium is decidedly the more important of these two, but a consideration of the ash of certain tissues of which the permeability has been much studied, points to a different conclusion. Abderhalden, for instance, finds that the blood corpuscles of a number of the domesticated mammals contain small and rather variable quantities of Mg, but no Ca.[8] And Katz has shown that samples of striated muscle taken from the human being, pig, ox, calf, deer, rabbit, dog, cat, hen, frog, haddock, eel, and pike contain larger and more constant quantities of Mg than of Ca. The Mg content of the muscle in this series of animals varies from 0.017 per cent of the weight of the fresh tissue in the haddock to 0.037 per cent in the hen; while the Ca content varies from 0.002 per cent in the ox to 0.04 per cent in the pike.[9]

[5] Loeb: Biochem. Zeitschr., 1911, xxxii, 308.
[6] Osterhout: Science, N. S., 1911, xxxiv, 187.
[7] Osterhout: Science, N. S., 1912, xxxv, 112; ibid., xxxvi, 350.
[8] Abderhalden: Zeitschr. f. physiol. Chem., 1898, xxv, 106.
[9] Katz: Arch. f. d. gesammt. Physiol., 1896, lxiii, 1.

An interesting observation which indicates that magnesium phosphate is present in considerable quantities at the surfaces of the striated muscle fibers is recorded by Hürthle.[10] He finds that if fresh frog's muscle fibers are treated with ammonia, they "cover themselves" with crystals of magnesium ammonium phosphate. It is difficult to see how such crystals can be formed, unless magnesium phosphate is previously present.

In view of the facts given above, it has seemed to me worth while to make a study of the osmotic properties of calcium and magnesium phosphate. I had hoped to make a more or less extended comparison between the permeability of these precipitates to various substances and that of living cells. But some two years of work on this subject have convinced me that the time is not yet ripe for such a comparison. One begins by thinking of a semi-permeable membrane as a more or less simple and stable kind of filter by means of which water can readily be separated from most substances that can be dissolved in it. But this view is far from the truth. The studies that have been made on the copper ferrocyanide membrane, which is at present the best known semi-permeable membrane, show that its permeability varies greatly with the physical conditions under which it is formed and to which it is afterward subjected—with the electrical conditions at the time of its formation, with the time which elapses between its formation and the experiment, with temperature, with the presence or absence of electrolytes, and with the nature of the electrolytes which happen to be present.[11] If one wishes, therefore, to determine whether one osmotic membrane is more or less permeable to a given substance than another, one must be careful to control all these conditions. To do this in the case of artificial membranes formed in the laboratory is by no means easy; it is obviously impossible when one of the membranes to be studied is that of a living cell. The experiments to be reported, therefore, bear only in a general way on the question whether the semi-permeable properties of the phosphate membranes are similar to those of the surfaces of animal and plant cells. But they do throw some light on the semi-permeable properties of these precipitates and on certain fundamental questions regarding the nature of semi-permeable membranes and of osmotic reactions in general. I am compelled by external circumstances either to publish this work now or to lay it aside for an indefinite period, and these considerations impel me to publish it now, though the results are in many respects fragmentary and incomplete.

[10] Hürthle: Arch. f. d. gesammt. Physiol., 1903, C, 451.

[11] Morse: The osmotic pressure of aqueous solutions; Publication No. 198 of the Carnegie Institution of Washington, 1914, Chapter IV.

THE CONDITIONS ON WHICH DEPEND THE CRYSTALLIZATION OF CALCIUM AND MAGNESIUM PHOSPHATE

Morse believes that true semi-permeability is an attribute of colloids only, and gives many cogent reasons for holding this belief.[12] Experiences of my own, which will be described later, lead me to concur in this belief; and I have spent a considerable amount of time in inquiring under what circumstances the phosphates of calcium and magnesium fail to crystallize.

I have confined my inquiry to the orthophosphates and monohydrophosphates of the two metals, as the dihydrophosphates can exist only in acid solutions. Abegg describes the crystallization of both the orthophosphate [$Mg_3(PO_4)_2$] and monohydrophosphate [$MgHPO_4$]·of magnesium and of the monohydrophosphate of calcium [$CaHPO_4$].[13] With regard to $Ca_3(PO_4)_2$, however, he says,[14] "This amorphous precipitate has never been observed to become crystalline."

$CaHPO_4$ precipitates were prepared by mixing together equimolecular solutions[15] of $CaCl_2$ and K_2HPO_4; $Ca_3(PO_4)_2$ precipitates, by mixing solutions of $CaCl_2$ with solutions of K_2HPO_4 to which KOH had previously been added. The KOH and K_2HPO_4 mixtures were made by adding together equal portions of equimolecular KOH and K_2HPO_4 solutions.

It was found that under these conditions the $CaHPO_4$ crystallized[16] within twenty-four hours, while the $Ca_3(PO_4)_2$ never crystallized, though its precipitates were kept sometimes for more than four months.

Precipitates of $MgHPO_4$ and $Mg_3(PO_4)_2$ were prepared in a manner corresponding to that which has just been described, $MgCl_2$ being substituted for the $CaCl_2$. The rapidity with which both these precipi-

[12] Morse: Loc. cit., pp. 87 and 209.

[13] Abegg: Handuch der anorganischen Chemie, Leipzig, 1905, vol. ii, Abt. 2, pp. 65 and 151.

[14] Ibid., p. 152.

[15] Concentrations are, throughout this article, given in the terms of what Morse calls "weight-normal solutions." See p. 479 of this article and "Osmotic pressure of aqueous solutions," Chapter V.

[16] It was determined whether or not the various precipitates crystallized by examining them microscopically. Such examination cannot, of course, show whether or not the precipitates are in a colloidal state in the sense of Morse, and capable of showing true semi-permeability. Morse finds, for instance, that precipitates of zinc ferrocyanide may become granular and lose their semi-permeable properties without becoming actually crystalline (Osmotic pressure of aqueous solutions, p. 91). But it may at least be said that crystalline precipitates do not exhibit semi-permeability.

tates crystallized was extremely variable. The $MgHPO_4$ sometimes crystallized in four days, and sometimes failed to crystallize at all, though its precipitates were kept for more than four months. I did not make any study of the conditions which control the crystallization of this precipitate; as it is so soluble that it can hardly be supposed to play a part in modifying the semi-permeable properties of living cells.

With regard to $Mg_3(PO_4)_2$ it was found that its crystallization depended on a number of conditions, of which the following were more or less extensively investigated. (1) Amount of alkali present. (2) Temperature. (3) Presence or absence of Ca. (4) Concentration of salts in supernatant fluid.

The influence of alkalinity on the crystallization of $Mg_3(PO_4)_2$ was investigated by mixing solutions of $MgCl_2$ and K_2HPO_4 together with varying quantities of KOH, and noting the time required for crystallization to occur (experiments 1 and 2). These experiments show that when varying proportions of $MgCl_2$, K_2HPO_4, and KOH are mixed together, the crystallization of the resulting precipitates depends on the relative proportions of the three ingredients. When the final mixture contains a higher molecular concentration of K_2HPO_4 than of KOH and enough Mg to combine with all the PO_4 as $Mg_3(PO_4)_2$, the resulting precipitates remain for the most part amorphous, for many weeks at least. But when the final mixture contains a higher molecular concentration of KOH than of K_2HPO_4 or not enough Mg to combine as $Mg_3(PO_4)_2$with all the PO_4 present, the resulting precipitate crystallizes more or less rapidly.

It is probably not correct to speak of the precipitates which have just been described as $Mg_3(PO_4)_2$. Such precipitates are probably mixtures of this compound with varying amounts of $MgHPO_4$ and $Mg(OH)_2$. There are many reasons for thinking that the quantities of the last two named substances present are small, but no special investigation of this question has been undertaken. For my purpose the main point of interest is the fact that magnesium phosphate combinations, which have a high degree of insolubility, may, under certain circumstances, remain amorphous indefinitely, or, at least, for a long time; for convenience the precipitates will in future be spoken of simply as *magnesium phosphate*.

Experiments 3 and 4 show the influence of temperature on the crystallization of magnesium phosphate. When formed above a certain critical temperature, which is not far from 23° the precipitates fail to crystallize, whereas they crystallize readily at lower temperatures. These

experiments indicate also that precipitates which have been formed at temperatures above 23° and have therefore failed to crystallize, show little or no tendency to crystallize, when later kept at lower temperatures. A comparison of experiment 3 with experiment 4 shows finally that, other things being equal, the precipitates have a greater tendency to crystallize when formed from more concentrated solutions. The precipitate formed from the less concentrated solutions at 20° in experiment 3 was only about half crystalline after 21 hours, while that formed from the more concentrated solutions in experiment 4 was completely crystalline at the end of the same period at the same temperature.

Experiment 5 shows that calcium has an inhibiting effect on the crystallization of magnesium phosphate precipitates.

THE OSMOTIC PROPERTIES OF CALCIUM AND MAGNESIUM PHOSPHATE AND OF COPPER FERROCYANIDE

The osmotic properties of calcium and magnesium phosphate and of copper ferrocyanide have been studied by making small, unsupported membranes of them according to the method of Traube[17], and by precipitating them on porous clay cups. The electrolytic method of precipitation of Morse[18] has not been employed in these experiments. It is, of course, highly desirable that the properties of phosphate membranes precipitated by the electrolytic method should be studied, but it would be out of the question to follow the technique of Morse in the rough preliminary survey which constitutes the subject matter of this article.

EXPERIMENTS WITH PHOSPHATE AND FERROCYANIDE MEMBRANES PRECIPITATED ON POROUS CLAY CUPS

Experimental methods

It has been my plan to determine as far as possible for what substances the phosphate membranes are impermeable and for what substances they are permeable, and to compare the rapidities with which substances of the latter class diffused through the membranes. To do this, it is not necessary to measure the osmotic pressure of the solutions used. The substance, of which the osmotic properties are to be studied,

[17] Traube: Arch. f. Anat., Physiol., und wissensch. Med., 1867, xxxiv, 123, 133, et seq.

[18] Morse: Loc. cit., 83–84.

may be precipitated on the inner surface of a porous cup, which may then be provided with suitable attachments for determining how rapidly fluid passes through it to the interior against a slight hydrostatic pressure. The cup is then filled with a solution of known composition and concentration and immersed for a given period in distilled water or in another known solution. At the end of the experimental period it is determined how much fluid has passed through the walls of the cup and the membrane; and the fluids within and without the cup are analyzed to determine how much, if any, of the solute experimented with has passed through the membrane from within the cup to the exterior.

I supposed at first that almost any kind of porous earthenware vessel would do for experiments of this class, and I spent a good deal of time in attempting to precipitate phosphate membranes and membranes of $Cu_2Fe(CN)_6$ on alundum filters and on various other kinds of porous clay vessels which allow a ready passage of water through their walls, but are supposed to hold back undissolved solids. I found, however, that all of the precipitates mentioned are easily forced through the walls of most of these vessels under pressures of a metre of water or less.

The porous cups which I finally found satisfactory were furnished me through the kindness of Prof. B. E. Livingston of the Johns Hopkins University. They are the regular cylindrical atmometer cups supplied by the *Plant World*, Tucson, Arizona; and are formed by pouring liquid kaolin and quartz mixture into dry plaster of paris moulds, and subsequently drying and burning the cups so formed. These cups are about 13 cm. high with an inner diameter of about 2.2 cm. and entirely unglazed. For the purposes of my experiments I filled the pores of the upper rims (about 2 cm. in height) with paraffin. This left an inner surface of about 70 sq. cm. on which the membrane was precipitated. The walls of the cup are about 0.25 cm. thick. These cups were connected by means of rubber attachments to an upper piece of glass provided with a stop-cock and an upright glass tube about one metre high. The whole cell so formed held from 70 to 80 cc. of fluid. The amount of fluid passing through the walls of the cup and the semi-permeable membrane was determined by noting the rise or fall of the meniscus in the upright tube. I used rubber attachments instead of the sealing wax used by Pfeffer[19] in his experi-

[19] Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 5 et seq.

ments, because I wished to work with alkalies in many cases, and because it was not necessary for me to make arrangements to withstand any considerable pressure. I need not describe my attachments in detail. They are such as will readily suggest themselves to anyone seriously interested in the subject. But it may be worth while to say that I found it much harder than I had supposed to entirely prevent leakage through rubber attachments, and overcame this difficulty only by using pretty tight fittings and binding the joints tightly with stout cotton thread which had previously been wet.

I removed the air from the porous cups by allowing first boiled distilled water and then a solution of one of the membrane-formers to seep through them under a pressure of about 70 cm. of water. Each of these fluids was allowed to seep through for a period of three or four days. After the cup had been thoroughly infiltrated with the outer membrane-forming solution, it was emptied of this fluid, rinsed out quickly with distilled water, dried slightly, filled with the inner membrane-forming solution and allowed to stand for twenty-four to forty-eight hours in the outer membrane-forming solution, there being no difference in the pressures within and without the cup. Subsequently a pressure of about 70 cm. of water was put on the inner membrane-forming solution, and the cup was allowed to stand for another twenty-four to forty-eight hours in the outer solution. In this way the semi-permeable membrane was precipitated on its inner surface.[20]

RESULTS OF THE EXPERIMENTS

I had little difficulty in making by these methods membranes of $Cu_2Fe(CN)_6$ which showed osmotic activity. Experiment 6 shows the behavior of such a membrane under various conditions, and experiment 7 gives the results of somewhat similar procedures with another copper ferrocyanide membrane.

These two experiments add a little to the already existing evidence for the view that the $Cu_2Fe(CN)_6$ membrane is impermeable for sugar[21] and highly permeable for NaCl.[22] Perhaps their most interesting result, however, is the rapid osmotic intake of fluid caused by the NaCl solution in spite of the facts that its osmotic pressure (as calculated from freezing point determinations) was nearly the same as that of

[20] Compare Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 4 et seq.

[21] Morse: Loc. cit., pp. 92 and 93.

[22] Traube: Arch. f. Anat. Physiol. und wissensch., Med., 1867, xxxiv, 137–141.

the sugar solution used, and that it escaped so rapidly from inside the cell to the exterior. In the first experiment the osmotic intake caused by the NaCl was nearly twice as rapid as that caused by the sugar; and, as the NaCl period fell between two sugar periods in which the osmotic intake was nearly the same, it can hardly be supposed that the result was due to any irreversible change taking place in the membrane. In the second experiment the osmotic intake caused by the NaCl was slower than that caused by the sugar, but it seems to me reasonable to explain this as caused by the decidedly greater leakiness of the second membrane.

For the experiments with membranes of $Ca_3(PO_4)_2$ the cups were provided with the same fittings as in the experiments which have just been described, and subjected to the same preliminary treatment. They were then impregnated with a 0.09M $CaCl_2$ solution and filled with a 0.075M K_2HPO_4 + 0.075M KOH solution, the details of the procedure being the same as in the case of the ferrocyanide membrane. Experiments 8 and 9 show that semi-permeable membranes can be made of $Ca_3(PO_4)_2$, and give an idea of the permeability of this membrane with respect to cane sugar and potassium hydroxide. The membrane is impermeable to cane sugar, but quite permeable to potassium hydroxide.

An interesting result of experiment 8 is shown in the period from March 13 to March 24. In the first four days of this period the cell contained a 0.22M $C_{12}H_{22}O_{11}$ solution; in the next three days, a KOH solution of about the same calculated osmotic pressure; and in the last four days, the same sugar solution as at first. It was immersed during the whole period in a 0.009M $CaCl_2$ solution. The cell showed considerable osmotic activity in both the first and second sugar periods, fluid passing to the interior against a moderate hydrostatic pressure. But during the alkali period there was practically no osmotic activity after the first hour, in spite of the fact that the alkali solution had about the same calculated osmotic pressure as the sugar solution. It is true that the alkali escaped fairly rapidly from the cell to the exterior. But the rate of its escape was not many times greater than that of the NaCl in experiment 6 between the dates March 16 and 20, and yet the NaCl solution caused a more rapid intake of fluid in this case than did a sugar solution of the same calculated osmotic pressure. Further, the results obtained in experiment 11 between November 3 and 11 show that a sodium chloride solution may cause greater osmotic activity in a mag-

nesium phosphate membrane than a sugar solution of the same calculated osmotic pressure, in spite of the fact that in this case also the salt escapes to a considerable extent through the membrane while the sugar does not. KOH does not cause the crystallization of the $Ca_3(PO_4)_2$ membrane, nor does it produce any known chemical change in it; the membrane is formed in the presence of a pretty strongly alkaline solution. Further, the fact that the membrane showed decided osmotic activity in the presence of a sugar solution subsequent to its treatment with the alkali indicates that this latter produced no destructive irreversible change in it. It seems likely, therefore, that alkalies and salts produce entirely different osmotic responses when applied to the surface of a semi-permeable membrane. It would be easy to find some explanation for this difference if it may be supposed that all such membranes are colloidal in nature, but difficult on any other basis.

Experiment 9 shows the same sort of result with sugar on the $Ca_3(PO_4)_2$ membrane as experiment 8; and, in addition, the result of filling the cell with a mixture of KCl and KOH. A rather slow intake of fluid is produced. Several features of the alkali effects shown in experiment 8 are reproduced, but it would be rash to attempt a detailed interpretation of these results at present.

Experiments 10, 11, and 12 show some of the properties of the magnesium phosphate membrane; the general method of making these membranes was the same as in the case of the ferrocyanide and calcium phosphate membranes. These experiments show that magnesium phosphate may retain its osmotic activity for two months or more in the absence of its membrane formers; that it is quite impermeable to cane sugar; somewhat permeable to KCl and NaCl; and apparently about equally so to each of the two salts. It seems to be decidedly less permeable to either NaCl or KCl than is the copper ferrocyanide membrane. It is highly permeable to ethyl alcohol.

In addition, two interesting points come out which bear on the nature of osmotic action in general. In experiment 11 the results obtained between November 3 and 11 indicate that a sodium chloride solution sets up a greater osmotic activity in the magnesium phosphate membrane than does a cane sugar solution of the same calculated osmotic pressure, in spite of the fact that the salt escapes through the membrane fairly rapidly and the sugar not at all. This result is similar to that obtained in experiment 6 in the case of the $Cu_2Fe(CN)_6$ membrane (see page 464); it indicates that solutions of neutral electrolytes have the

general property of setting up a more rapid osmotic action in semi-permeable membranes than solutions of non-electrolytes of the same calculated osmotic pressure.

The results obtained in experiment 11 from November 3 to 7, 11 to 20, 1914, and from November 25, 1914 to February 12, 1915 indicate that the rapidity with which NaCl escapes through the magnesium phosphate membrane depends, among other things, on the concentration of the outer solution. During these periods the salt uniformly escaped more rapidly when the outer solution was more dilute.

In regard to the experiments on the osmotic properties of magnesium phosphate, it must be added that there were a number of other attempts to precipitate semi-permeable membranes of this substance on porous cups, besides the three which are described in the protocols. In some of these attempts the precipitations were carried out at temperatures below 23°; in others, the proportions of alkali added to the phosphate solutions were such as would induce crystallization of the magnesium precipitates. It was found that the precipitates formed under these conditions showed no osmotic activity. The experiments with magnesium phosphate taken all together, therefore, are in accord with the view that precipitates must be in a colloidal condition if they are to exhibit osmotic acitivty.

EXPERIMENTS WITH UNSUPPORTED MEMBRANES

Traube studied the osmotic properties of a number of substances by precipitating thin layers of them across the mouths of small glass tubes. He filled tubes, for instance, with solutions of $K_4Fe(CN)_6$, and immersed the lower open ends in solutions of copper salts. Under these circumstances a layer of $Cu_2Fe(CN)_6$ forms across the open mouth of the tube. I have attempted to form magnesium phosphate membranes in a similar way, but without any very satisfactory results. If a small glass tube be filled with a solution of K_2HPO_4 and KOH and dipped into a solution of $MgCl_2$, a layer of magnesium phosphate is, of course, precipitated across its mouth. But this layer rapidly becomes thicker, and the least disturbance suffices to detach it from the walls of the tube; so that it cannot be used even for the rough kind of osmotic experiment described by Traube.

Membranes of $Ca_3(PO_4)_2$, however, produced in this way are quite as resistant and satisfactory for experimentation as the $Cu_2Fe(CN)_6$ membranes. To work with such membranes it is necessary in the first

place to use a rather small glass tube. Further, the phosphate and calcium solutions used in making the membrane must have somewhere near the same osmotic pressure; otherwise, the membrane will be very soft, and will rapidly become thicker.

In my experiments I have used tubes which had an inner cross sectional area of about 0.14 sq. cm. (inner diameter, about 0.42 cm.). These were closed at the top with a rubber tube and pinch cock; the lower ends were inserted through bored corks into small cylindrical vials.[23] In some experiments a few cubic centimeters of 0.186M K_2HPO_4 + 0.186M KOH[24] were placed in the vials and the lower ends of the tubes were filled with 0.666M $CaCl_2$ solution. Under these circumstances there is a considerable tendency for fluid to pass through the membrane from the phosphate to the calcium solution. As the calcium solution has a decidedly higher calculated osmotic pressure than the phosphate solution, there can be little doubt that this phenomenon is the result of osmotic action on the part of the calcium phosphate membrane.

It is perhaps not generally realized by biologists that impermeability to NaCl and KCl is by no means a usual characteristic of semi-permeable membranes. Traube[25] asserts that a $Cu_2Fe(CN)_6$ membrane when infiltrated with AgCl becomes impermeable to KCl. But he finds the $Cu_2Fe(CN)_6$ membrane by itself highly permeable to KCl and probably also to NaCl (loc. cit., pp. 137–141). Morse[26] describes experiments in which KCl solutions were allowed to act on electrolytically deposited $Cu_2Fe(CN)_6$ membranes. He found that old (and probably thick) membranes at first exhibited a high resistance to the passage of KCl, but that the salt had a tendency to render the membrane permeable to itself.

In experiments of my own I have found the $Cu_2Fe(CN)_6$ membrane highly permeable both to KCl and to NaCl. In order to compare the permeability of this membrane to NaCl with that of the $Ca_3(PO_4)_2$ membrane, the following experimental procedure was employed. Four vials, and four glass tubes with inner diameters of 0.42 cm. were arranged as described above. Into the lower end of each of the tubes was drawn 0.28 cc. of 0.125M $Na_4Fe(CN)_6$ + 0.5M NaCl solution:

[23] Compare Traube: Arch. f. Anat., Physiol. u. wiss. Med., 1867, xxxiv, 123 and 133.

[24] Made up by adding 32.4 grams K_2HPO_4 and 10.4 grams KOH to liter of water.

[25] Traube: Loc. cit., p. 146.

[26] Morse: Loc. cit., pp. 211, et seq.

and 10 cc. of 0.25M $CuSO_4$ solution was placed in each of the vials. The lower ends of the tubes containing the chloride and ferrocyanide mixture were then immersed in the copper solution, and 0.14 cc. of the chloride and ferrocyanide mixture was forced through the lower end of the tube and appeared below it as a drop covered by the $Cu_2Fe(CN)_6$ membrane. This drop was then drawn back into the tube carrying the membrane behind it and finally leaving the latter in a much folded state across the tube's mouth.[27] The tubes were then immediately withdrawn from the vials and the solution left in the latter was analyzed for Cl. The amount found was taken to represent that which escaped from the inner solution during the formation of the membranes. The same procedure was then exactly repeated up to the point at which the drop of chloride and ferrocyanide mixture was drawn back into the tubes, leaving the folded membrane across its mouth. At this point the tubes, instead of being withdrawn, were left for twenty minutes with their lower ends immersed in the $CuSO_4$ solution. As the chloride and ferrocyanide mixture has a decidedly higher osmotic pressure than the $CuSO_4$ solution, fluid passed from the latter to the former during this period, more rapidly at first and more slowly afterward; at the end of the twenty minutes the membrane was completely distended and filled with a drop of diluted chloride and ferrocyanide mixture, about 0.14 cc. of fluid having passed through it during the interval. The drops of chloride and ferrocyanide mixture were now again drawn back into the tubes, the tubes were withdrawn from the $CuSO_4$ solution, and this last was analyzed for Cl. In both cases, of course, the Cl must represent NaCl which has escaped from the chloride and ferrocyanide mixture.

It was found in the first case when practically no time was allowed for diffusion that the 40 cc. of $CuSO_4$ solution contained 0.0015 gram of NaCl. In the second case, after the diffusion had gone on for twenty minutes the 40 cc. of $CuSO_4$ solution contained 0.0115 gram of NaCl. It may be supposed that 0.0115 minus 0.0015 or 0.01 gram of NaCl diffused through the four $Cu_2Fe(CN)_6$ membranes in the course of twenty minutes. The four glass tubes contained originally 0.28×4 or 1.12 cc., of 0.125M $Na_4Fe(CN)_6$ + 0.5M NaCl solution or 0.03248 gram of NaCl. Of this 0.0015 gram was lost during the formation of the membrane, leaving 0.031 gram at the time the diffusion began.

[27] Compare Traube: Arch. f. Anat., Physiol. u. wiss. Med., 1867, xxxiv, pp. 136 and 137.

And of this 0.031 gram 0.01 gram or about 32 per cent diffused through the four membranes in the course of twenty minutes.

A similar experiment was carried out to test the permeability of the $Ca_3(PO_4)_2$ membrane to NaCl. In this case the four tubes were filled each with 0.28 cc. of 0.24M $CaCl_2$ + 0.375M NaCl solution and immersed in 10 cc. of 0.186M K_2HPO_4 + 0.186M KOH solution. It was found that under these circumstances it required about two hours for as much fluid to pass through the $Ca_3(PO_4)_2$ membranes as passed through the $Cu_2Fe(CN)_6$ membranes in the previously described experiment in twenty minutes. In this case, therefore, the diffusion was allowed to go on for two hours instead of for twenty minutes; otherwise the procedure was the same as in the previously described experiment.

When practically no time was allowed for diffusion the 40 cc. of 0.186M K_2HPO_4 + 0.186M KOH solution contained 0.0021 gram NaCl. When the diffusion was allowed to go on for two hours, the 40 cc. of alkaline phosphate solution contained 0.0034 gram NaCl. It may be supposed therefore that 0.0013 gram of NaCl diffused through the four $Ca_3(PO_4)_2$ membranes in the course of two hours. The four tubes of 0.24M $CaCl_2$ + 0.375M NaCl solution contained originally 0.0243 gram of NaCl, and of this 0.0021 gram was lost during the formation of the membranes leaving 0.0222 gram at the time the diffusion began. 0.0013 is about 5.8 per cent of 0.0222, and it appears, therefore, that only about 5.8 per cent of the NaCl contained in the four tubes diffused through the $Ca_3(PO_4)_2$ membranes in the course of two hours. That is about five times as much NaCl diffused through the $Cu_2Fe(CN)_6$ membrane in twenty minutes as diffused through the $Ca_3(PO_4)_2$ membrane under more or less similar conditions in two hours. I have confirmed these results by other experiments which it is not necessary to describe in detail, and I am prepared to assert, therefore, that, under the conditions of formation which have been described, the $Cu_2Fe(CN)_6$ membrane is much more permeable to NaCl than the $Ca_3(PO_4)_2$ membrane.

PROPERTIES OF THE CELLOIDIN MEMBRANE

In my earliest experiments I used celloidin membranes on which layers of calcium phosphate were precipitated. Celloidin or collodion membranes have been used for many years for the study of osmotic phenomena; Smith[28] has suggested their use as a basis for the precipi-

[28] Smith: Science, 1913, N. S., xxxvii, 379.

tation of other forms of semi-permeable material. Since Traube's time it has been known that the permeability of sheets of celloidin varied with the circumstances of their formation. Such sheets are formed by pouring an alcohol-ether solution of celloidin on a flat surface, allowing the alcohol and ether to evaporate to a certain extent, and then immersing the sheet so formed in water. If the water be applied before much of the alcohol and ether have evaporated, the membrane will be found highly permeable; otherwise, it becomes quite impermeable to water as well as to dissolved substances.[29]

I formed my celloidin membranes by pouring the alcohol-ether solution over the inner surface of a beaker, and carried out several experiments to determine the properties of the membranes so formed under different conditions of drying. The celloidin sacs were tied around large rubber corks, and the cells so formed were fitted with long upright outlet tubes, filled with various solutions, and immersed in others. When the celloidin membrane was immersed in water less than an hour after its formation it was highly permeable to water and dissolved salts; it was easy to show that a solution of K_2HPO_4 could be rapidly forced through its walls by the very moderate hydrostatic pressure of 50 to 100 cm. of water. But if the membrane was not wet with water until after the alcohol-ether mixture had dried out for twenty-four hours or more, it became highly impermeable, though it showed some osmotic activity. The following experiment shows this. A celloidin membrane was allowed to dry for twenty-four hours, and then washed for twenty-four hours in tap water. At the end of this time it was used in making a cell, which was filled with 0.075M K_2HPO_4 and immersed in distilled water. The contents of the cell were put under a pressure of 78 cm. of water. The meniscus immediately began to rise in the outlet tube and continued to do so steadily for the twenty days during which the experiment was continued at a rate indicating that a little more than 0.1 cc. of fluid per day was passing into the cell.[30] At the end of the twenty days the water in which the cell had been immersed was tested for K_2HPO_4 and found to contain none.[31] In another similar experiment, similar results were obtained.

In still another experiment a membrane was used which had been

[29] See Traube: Arch. f. Anat. Physiol., und wissensch. Med., 1867, xxxiv, 106

[30] The area of semi-permeable surface was involved in this experiment was about 60 sq. cm.

[31] The escape of 1 per cent of the K_2HPO_4 originally contained in the cell could easily have been detected by means of the test used.

dried for somewhere between one and twenty-four hours before being wet with water. The cell made with this membrane was filled with 0.075M K_2HPO_4 solution and immersed in distilled water. The meniscus immediately began to rise in the outlet tube, indicating a rapid intake of fluid; about 2 cc. passed through the membrane[30] into the cell in the first half hour. In the next two hours only about 1.5 cc. of fluid passed into the cell, and at the end of that time a good deal of K_2HPO_4 was found to have escaped to the distilled water surrounding it.

These experiments seem to me interesting, because they show so clearly that a given chemical substance may have enormously different semi-permeable properties under different physical conditions. It was found possible to alter the osmotic properties of the celloidin membrane by precipitating calcium phosphate on it; but, as it was not possible accurately to control the variable osmotic properties of the celloidin itself, the experiments carried out along these lines are of very doubtful significance, and I do not consider it worth while to give any description of them.

DISCUSSION

The colloidal character of semi-permeable membranes and the nature of osmotic action

The results of the experiments described in the preceding pages lend support to the two hypotheses advanced by Morse—the hypotheses, namely, that semi-permeable membranes are always colloidal, and that the passage of water through such membranes is the result of unequal hydration at the opposite surfaces of the colloid.

The first of these hypotheses is supported by the facts that a membrane highly impermeable to K_2HPO_4 and yet showing some osmotic activity can be made of an undoubted colloid, celloidin (pp. 469–471); that semi-permeable membranes can readily be made of $Ca_3(PO_4)_2$, which never crystallizes (p. 459 and pp. 464, 465) that semi-permeable membranes can be made from magnesium phosphate only under such conditions as prevent its crystallization (p. 466); and that the semi-permeable properties of the various membranes are altered by such influences as would be likely to change the physical state of colloids (pp. 464–466, and pp. 469–471). Finally this first hypothesis is supported by whatever evidence tends to support the second, for the view that osmotic action depends on unequal hydration at the two surfaces of a colloid membrane presupposes the existence of a colloid membrane to start with.

The hypothesis that the passage of water through a semi-permeable membrane is the result of unequal hydration of the two surfaces is supported by the anomalous results obtained with regard to the amount of osmotic activity set up by salt, sugar, and potassium hydroxide solutions. A sodium chloride solution causes a more rapid passage of water through the ferrocyanide and magnesium phosphate membranes than does a cane sugar solution of the same calculated osmotic pressure, while an equally concentrated potassium hydroxide solution causes practically no osmotic activity in the calcium phosphate membrane. The salt and alkali escape through the membranes with considerable rapidity, while the sugar does not escape at all (pp. 464–466). If the hypothesis just stated is correct, these facts might be explained as the result of the well known rule that neutral electrolytes have a greater dehydrating effect on colloids than non-electrolytes while alkalies tend to increase their power of holding water; I do not see at present how they can be explained on any other basis.

Certain experiments reported many years ago by Graham[32] are interesting in this connection. Graham found that the osmotic activity set up in a piece of pig's bladder by sugar solutions was very small in comparison to that set up by salt solutions, and that when the bladder separated a dilute solution of acid from distilled water, a considerable quantity of fluid passed through the membrane from the acid solution to the other side. Graham's results with the sugar and salt solutions are similar to mine, though not so clear cut, because the salt solutions used by him had a much higher calculated osmotic pressure than the sugar solutions. The result with the acid, however, is very striking. It seems quite inexplicable from the ordinary conceptions of osmosis and osmotic pressure, but can be readily explained on the hydration hypothesis from the well known fact that acids tend to hydrate colloids even more strongly than pure water.

If semi-permeable membranes are really hydrated colloids, it seems probable that their semi-permeable properties would vary with the degree of their hydration. My experiments furnish evidence for the view that this is the case, and also for the view that the membranes become more readily permeable both to water and dissolved substances the greater the degree of their hydration. Perhaps the most direct evidence for this view is the series of experiments with celloidin membranes

[32] Quoted by Girard: Journ. d. Physiol. et de path. générale, 1911, xiii, pp. 365–367.

(pp. 469–471). But on p. 466 it is shown that the magnesium phosphate membrane also is more permeable to salts when it is in contact with a more dilute outer solution and, therefore, presumably in a state of greater hydration.

Much of the peculiar behavior of living cells would find a ready explanation from the considerations above set forth and from the additional consideration that in the case of living cells we are dealing probably always with leaky membranes. The experiments of Morse on the osmotic pressure of potassium chloride and of lithium chloride[33] show that we have yet much to learn regarding the osmotic behavior of electrolytes even with respect to the most ideal semi-permeable membranes that have yet been produced. The conditions become much more complicated; and the results, correspondingly less predictable, when we have to deal with such membranes as may be supposed to cover the surfaces of living cells.

DO MAGNESIUM AND CALCIUM PHOSPHATE PLAY ANY PART IN THE OSMOTIC PROPERTIES OF LIVING TISSUES?

It is well known that calcium, magnesium, and inorganic phosphates are constant or nearly constant constituents of living tissues; and there is much evidence (reviewed at the beginning of this article) for the view that calcium, at least, plays a very important physiological role, and has the property of rendering the surfaces of cells less permeable for other ions. The experiments reported in this article are at least sufficient to show that both calcium and magnesium phosphate are capable of forming semi-permeable membranes.

Beyond this, however, the experiments do not go very far in showing either that these substances do or do not play an important part in giving to living cells their semi-permeable properties. It is hardly possible that they should, because we know at present so little, on the one hand, of the influences which affect the permeability of osmotic membranes, and, on the other, of the conditions which obtain in living cells. Still, it seems worth while to review very briefly those of the results which bear on this question.

Both the calcium and magnesium phosphate membranes are highly impermeable to cane sugar, potassium phosphate, calcium chloride, and magnesium chloride. Under certain conditions they may show a con-

[33] Morse: Loc. cit., Chapter XI.

siderable degree of impermeability to sodium chloride and potassium chloride. The calcium phosphate membrane is permeable to potassium hydroxide, when that substance is present in considerable quantities. The magnesium phosphate membrane is quite permeable to ethyl alcohol.

The surfaces of living cells are commonly more or less impermeable to cane sugar and to the salts mentioned above. They are always, so far as I know, quite permeable to ethyl alcohol. Most cells are killed by potassium hydroxide when it is present in any considerable quantity, and, at the same time, their surfaces become permeable to it as well as to crystalloids generally. Striated muscle cells and kidney cells are apparently decidedly more permeable to potassium chloride than to sodium chloride.[34] In this respect they seem to differ from the magnesium phosphate cells. But the surfaces of red blood corpuscles are equally impermeable to both these salts.[35] These facts and a great many others[36] show how complicated is the question of the permeability of living cells.

It is perhaps just to close this part of the discussion by saying that the semi-permeable properties of magnesium and calcium phosphate, so far as they have been studied, are as much like those of living tissues as are the semi-permeable properties of any other artificial osmotic membranes that have been examined up to this time.

In conclusion I wish to extend my most heartfelt thanks to Dr. John Marshall and to the staff of the Hare Chemical Laboratory of the University of Pennsylvania, without whose kind assistance and support the work reported in the foregoing article would have been difficult or impossible.

SUMMARY

1. Semi-permeable membranes can be formed both from calcium and from magnesium phosphate.

2. In order to form semi-permeable membranes from magnesium phosphate, it is necessary to precipitate it under such conditions that it does not crystallize.

[34] Siebeck: Arch. f. d. gesammt. Physiol., 1912, cxlviii, 443; 1914, cl, 316. Meigs: Journ. Exper. Zool., 1912, xiii, pp. 518 520.

[35] Hamburger: Osmotischer Druck und Ionenlehre, Wiesbaden, 1902, vol. 1, pp. 208 and 209.

[36] The surfaces of human red blood cells and of these of certain other animals, for instance, appear to be quite permeable to dextrose; see Kozawa: Biochem. Zeitschr., 1914, lx, 231; Masing: Arch. f. d. gesammt. Physiol., 1914, clix, 476.

3. The calcium phosphate membrane is impermeable or nearly impermeable to cane sugar, dipotassium phosphate and calcium chloride, only slightly permeable to sodium chloride, and quite permeable to potassium hydroxide.

4. The magnesium phosphate membrane is impermeable or nearly impermeable to cane sugar, dipotassium phosphate and magnesium chloride, somewhat permeable to sodium and potassium chloride, and highly permeable to ethyl alcohol.

5. Anomalous results are obtained when the calcium phosphate, magnesium phosphate, and copper ferrocyanide membranes are subjected to the action of neutral electrolytes and alkalies. Sodium chloride solutions cause a more rapid osmotic action in the copper ferrocyanide and magnesium phosphate membranes than do cane sugar solutions with the same calculated osmotic pressure, in spite of the fact that the salt escapes through the membrane to a considerable extent, while the sugar does not. Potassium hydroxide produces no lasting osmotic activity in the calcium phosphate membrane, though it apparently causes no marked irreversible change in the membrane, and does not escape through it very much faster than does sodium chloride through the copper ferrocyanide membrane.

6. It is shown that the semi-permeable properties of celloidin membranes depend to a very great extent on their physical state, and reasons are given for thinking that this is true of semi-permeable membranes in general.

7. Evidence, in addition to that already given by Morse, is adduced for the view that semi-permeable membranes are always colloidal, and that the passage of water through them is the result of unequal hydration of the colloid at its two surfaces.

Experiment 1

12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 0.8 cc. 0.86M KOH	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 1.6 cc. 0.86M KOH	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 2.0 cc. 0.86M KOH
12.05 p.m., May 2. Precipitate is now almost entirely crystalline	12.15 p.m., May 2. Precipitate contains a few crystals, but is still for the most part amorphous	12.15 p.m., May 2. Precipitate is still entirely amorphous	12.15 p.m., May 2. Precipitate is now chiefly made up of crystals
11.40 a.m., June 6. Precipitate now entirely crystalline.	11.50 a.m., June 6. Precipitate contains a good many crystals, but is still, for the most part, amorphous	12.00 m., June 6. Precipitate still entirely amorphous	12.00 m., June 6. Precipitate now almost entirely crystalline

Experiment 2

2.53 p.m., Oct. 14, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 5 cc. 0.2M $MgCl_2$ and 0.5 cc. 0.86M KOH	3.00 p.m., Oct. 14, 1914. 5 cc. 0.15M K_2HPO_4 mixed with 10 cc. 0.2M $MgCl_2$ and 0.5 cc. 0.86M KOH
3.55. Precipitate contains a few small crystals, but is still, for the most part, amorphous	4.00. Precipitate still entirely amorphous.
10.30 a.m., Oct. 15. Precipitate is still largely amorphous but contains a good many crystals	10.35 a.m., Oct. 15. Precipitate still entirely amorphous.
11.15 a.m., Oct. 16. Precipitate is now about half crystalline and half amorphous	11.15 a.m., Oct. 16. Precipitate still entirely amorphous

3.40 p.m., Dec. 4, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 10°	4.10 p.m., Dec. 1, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096 M KOH. Temp. 18°	12.45 p.m., Dec. 10, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°	11.50 a.m., Jan. 23, 1915. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 23°	2.54 p.m. Dec. 11, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 27°
11.27 a.m., Dec. 5. Precipitate entirely crystalline Temp. 10°	10.10 a.m. Dec. 2. Precipitate entirely crystalline. Temp. 18°	9.25 a.m., Dec. 11. Precipitate about half crystalline and half amorphous Temp. 20°.		10.30 a.m. Dec. 12, Precipitate still entirely amorphous. Temp. 21.5°
		10.20 a.m., Dec. 12. Precipitate is about two - thirds crystalline and one-third amorphous. Temp. 19°	9.30 a.m., Jan. 25. Precipitate contains a fair number of crystals, but is still, for the most part, amorphous. Temp. 14°	
				11.47 a.m. Dec. 15, Precipitate still entirely amorphous. Temp. 15°
		12.30 p.m., Feb. 19, 1915. Precipitate now entirely crystalline. Temp. 21° (1)	2.30 p.m., Feb. 19, 1915. Precipitate contains a good many crystals, but is still, for the most part, amorphous. Temp. 21° (1)	12.47 p.m. Feb. 19, 1915. Precipitate contains a few crystals, but is still, for the most part, amorphous. Temp. 21° (1)
		(1) Between Dec. 10, 1914, and Feb. 19, 1915, the temperature varied between 10° and 25°	(1) Between Jan. 25 and Feb. 19 the temperature varied between 10° and 25°	(1) Between Dec. 12, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°

Experiment 4

3.40 p.m., Dec. 4, 1915. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 10°	12.45 p.m., Dec. 10, 1914. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 20°	11.55 a.m., Jan. 23, 1915. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 23°	2.54 p.m., Dec. 11, 1914. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 27°
11.35 a.m., Dec 5. Precipitate entirely crystalline. Temp. 10°	9.30 a.m., Dec. 11. Precipitate entirely crystalline. Temp. 20°		10.32 a.m., Dec. 12. Precipitate still entirely amorphous. Temp. 21.5°
		10.10 a.m., Jan. 25. Precipitate contains a large number of crystals, but is still chiefly amorphous. Temp. 14°	
			11.53 a.m., Dec. 15. Precipitate still entirely amorphous. Temp. 15°
		2.33 p.m., Feb. 19, 1915. Precipitate contains a large number of crystals, but is still chiefly amorphous. Temp. 21° (1)	12.50 p.m., Feb. 19, 1915. Precipitate contains a few crystals but is still chiefly amorphous. Temp. 21° (1)
		(1) Between Jan. 23 and Feb. 19 the temperature has varied between 10° and 25°	(1) Between Dec. 12, 1914 and Feb. 19, 1915, the temperature has varied between 10° and 25°

Experiment 5

12.00–12.05 p.m., Dec. 7, 1914. 5 cc. 0.2M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°	12.00–12.05 p.m., Dec. 7, 1914. 5.15 cc. 0.19M $MgCl_2$ + 0.019M $CaCl_2$ mixed with 5.15 cc. 0.134M K_2HPO_4+0.096M KOH. Temp. 20°	12.00–12.05 p.m., Dec. 7, 1914. 5.8 cc. 0.17M $MgCl_2$ + 0.086M $CaCl_2$ + 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°
3.15 p.m. Precipitate entirely crystalline. Temp. 21°	3.22 p.m. Precipitate about half crystalline and half amorphous. Temp. 21°	3.25 p.m. Precipitate entirely amorphous. Temp. 21°
	10.00 a.m., Dec. 8. Precipitate about half crystalline and half amorphous. Temp. 17°	10.05 a.m., Dec. 8. Precipitate contains a very few crystals, but is still almost entirely amorphous. Temp. 17°
	2.20 p.m., Feb. 19, 1915. Precipitate about half crystalline and half amorphous. Temp. 21° (1)	2.22 p.m., Feb. 19, 1915. Precipitate contains a very few crystals, but is still almost entirely amorphous. Temp. 21° (1)
	(1) Between Dec. 7, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°	(1) Between Dec. 7, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°

EXPLANATION OF EXPERIMENTS 6 TO 12 INCLUSIVE

These experiments were carried out on membranes of $Cu_2Fe(CN)_6$, $Ca_3(PO_4)_2$ and magnesium phosphate (see p. 460) precipitated on the inner surfaces of such porous cups as are described on p. 462. The dimensions and capacity of the cups are given on p. 462. The preliminary treatment of the cups and the general method by which the membranes were precipitated are given on pp. 461 to 463. The succeeding protocols give a condensed history of the behavior of the osmotic cells from the period when the actual membrane formation was begun.

The solutions described are what Morse calls "weight-normal solutions" (Osmotic pressure of aqueous solutions, Chapter V). That is, a "molecular" solution of cane sugar would mean a solution made by adding 342 grams of cane sugar to a litre of water. In the same way,

a "0.5M NaCl + 0.5M KCl solution" would mean a solution made by adding 29.2 grams of NaCl and 37.2 grams of KCl to a litre of water.

The amounts of dissolved substances which passed through the walls of the cells were determined by using solutions of known strength at the beginning of the experiments, and making a chemical analysis of either the inner or the outer solution at the end. Which of these procedures was adopted in each particular case is shown in the individual protocols. In some cases both inner and outer solutions were analyzed; and it was then found, as was to have been expected, that more solute disappeared from the inner solution than could be recovered in the outer solution. In experiment 12, for instance, at the end of the period between January 28 and February 3, 1915, it was found that 0.18 gram NaCl had disappeared from the inner solution while only 0.04 gram was recovered in the outer solution. The 0.14 gram left unaccounted for was no doubt held in the membrane itself and in the pores of the earthenware cup.[37] The difference observed in this case was no doubt extreme. It would be much less, for instance, in experiment 6, March 16 to 20, where the membrane and pores of the earthenware cup already contained much NaCl when the experimental period in question was begun.

Sugar was determined by means of the saccharimeter. Under the conditions of the experiment this method made possible the determination in the outer solution of about 1 per cent of the sugar originally contained in the inner solution. Alcohol was determined by means of the pyknometer. The method adopted made possible the determination of the escape of about 0.5 per cent of alcohol from the inner solution. Sodium and potassium chloride were determined by the Vollhard-Arnold method of chlorine determination (see Hawk: Practical Physiological Chemistry, 3d edition, 1910, pp. 390–391); and potassium hydroxide, by titrating against HCl with azo-litmin as an indicator. These determinations are decidedly more accurate than either the sugar or alcohol determinations.

Most of the experiments were carried out at room temperature, which underwent considerable variations; and it was noted, of course, that changes in temperature caused changes in the position of the meniscus in the outlet tube independent of the osmotic changes of the quantity of fluid in the cell. The temperature changes, however, were small in comparison to the osmotic changes. Where small osmotic changes are recorded throughout the experiments, it may be taken for granted that the readings on which they depend were taken at the same temperatures.

[37] Compare Morse: Osmotic pressure of aqueous solutions, p. 213.

Experiment 6. February 3, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Feb. 5 to 6, 1914	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	0
Feb. 6 to 9	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	65
Feb. 9 to 17	0.05M $K_4Fe(CN)_6$	0.012M $CuSO_4$	0.5	Traces	80
Feb. 17 to 23	0.08M $K_4Fe(CN)_6$	0.012M $CuSO_4$	1.0	0	80
Feb. 23 to 26	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.22M $C_{12}H_{22}O_{11}$+ 0.007M $CuSO_4$	0.07	Not determined	50
Feb. 26 to Mar. 3	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	2.25	Not determined	65
Mar. 3 to 10	0.22M $C_{12}H_{22}O_{11}$+ 0.003 M $K_4Fe(CN)_6$	0.007M $CuSO_4$	4.7	0	130
Mar. 10 to 12	0.12M NaCl+ 0.003M $Na_4Fe(CN)$	0.12M NaCl+ 0.007M $CuSO_4$	−0.7[2]	Not determined	60
Mar. 12 to 16	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.2	Not determined	90
Mar. 16 to 20	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.8	14% of NaCl	130
Mar. 20 to 24	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	3.3	0	90

This experiment was carried out at room temperature which varied between 12° and 26°.

[1] The quantities of solute which escaped, were, throughout this experiment, determined by analyzing the outer solutions.

[2] 0.7 cc. fluid escaped from within the cell to the outer solution, as indicated by the minus sign.

Experiment 7. February 14, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Feb. 14 to 16, 1914	0.08M $K_4Fe(CN)_6$	0.22M $CuSO_4$	Not determined	0	3
Feb. 16 to 19	0.08M $K_4Fe(CN)_6$	0.22M $CuSO_4$	5.6	0	90
Feb. 19 to 20	0.08M $K_4Fe(CN)_6$	0.25M $CuSO_4$	0.17	0	65
Feb. 20 to 21	0.08M $K_4Fe(CN)_6$	0.05M $CuSO_4$	3.6	0	95
Feb. 21 to 23	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	0
Feb. 23 to 26	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.22M $C_{12}H_{22}O_{11}$+ 0.007M $CuSO_4$	−0.7[2]	0	40
Feb. 26 to Mar. 2	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	18.6	Not determined	120
Mar. 2 to 6	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	18.2	2.7% of $C_{12}H_{22}O_{11}$	120
Mar. 6 to 10	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.46	26.6% of NaCl	90
Mar. 10 to 14	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	17.0	0	100

This experiment was carried out at room temperature, which varied between 12° and 26°.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] 0.7 cc. fluid passed from within the cell to the outer solution, as indicated by the minus sign.

Experiment 8. March 9, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Mar. 9 to 11	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	Not determined	0	0
Mar. 11 to 13	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	−1.15[2]	0	53
Mar. 13 to 17	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4 0.0075M KOH	0.009M $CaCl_2$	3.9	0	100
Mar. 17 to 20	0.127M KOH+ 0.0075M K_2HPO_4	0.009M $CaCl_2$	0.1[3]	20% of KOH	60
Mar. 20 to 24	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.36[4]	0	65
Mar. 24 to 28	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.29	0	85

This experiment was carried out at room temperature, which varied between 15° and 26°.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] 1.15 cc. of fluid passed from within the cell to the outer solution as indicated by the minus sign.

[3] As a result of filling the cell with alkali, there was at first a quite rapid though short-lasting passage of fluid from without inward; 0.08 cc. passed in in the first half hour and 0.04 cc. in the second half hour. In the succeeding four days, however, there was a very slight loss of fluid from the cell.

[4] The cell showed osmotic activity *immediately* after the alkali was replaced by the sugar solution. 0.15 cc. of fluid passed in in the first hour; 0.42 cc. in the first 21 hours.

Experiment 9. March 11, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Mar. 11 to 13	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	Not determined	0	0
Mar. 13 to 16	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	−1.12[2]	0	53
Mar. 16 to 21	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.6	0	75
Mar. 21 to 25	0.086M KCl+ 0.032M KOH+ 0.0075M K_2HPO_4	0.009M $CaCl_2$	0.84[3]	20% of KOH	62
Mar. 25 to 28	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	0.9[4]	0	62

This experiment was carried out at room temperature, which varied between 15° and 26°.

[1] The quantities of solute which escaped, were, throughout this experiment, determined by analyzing the outer solutions.

[2] 1.12 cc. of fluid passed from within the cell to the outer solution as indicated by the minus sign.

[3] As a result of filling the cell with the alkaline solution, there was at first, in this case as in Experiment 8, an unusually rapid passage of fluid from without inward; 0.2 cc. of fluid passed in in the first hour and 0.07 cc. in the second hour.

[4] The cell showed osmotic activity in this case as in Experiment 8 immediately after the alkaline solution was replaced by the sugar solution.

Experiment 10. May 4, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CENTIMETRES OF WATER
May 4 to 6	0.2M $MgCl_2$	0.134M K_2HPO_4+ 0.092M KOH	Not determined	0
May 6 to 8	0.2M $MgCl_2$	0.134M K_2HPO_4+ 0.092M KOH	−1.12[1]	40
May 8 to June 8	0.2M $MgCl_2$	0.0134M K_2HPO_4+ 0.0092M KOH	10.3	75
June 8 to 29	0.2M $MgCl_2$	0.0134M K_2HPO_4+ 0.0092M KHO	12.0	100

This experiment was carried out at room temperature which varied between 18° and 29°. The passage of dissolved substances through the walls of the cell was not followed at all.

[1] 1.12 cc. fluid passed from within the cell to the outer solution, as indicated by the minus sign.

Experiment 11. October 29, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	PERCENTAGE OF SOLUTE WHICH ENTERED CELL FROM OUTER SOLUTION	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Oct. 29 to 30, 1914	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.095M KOH	Not determined	0	Not determined	4
Oct. 30 to Nov. 2	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.095M KOH	Not determined	0	Not determined	70
Nov. 2 to 3	0.3M $MgCl_2$	0.0134M K_2HPO_4+ 0.0095M KOH	0.33	0	Not determined	82
Nov. 3 to 7	0.12M NaCl+ 0.015M $MgCl_2$	0.0067M K_2HPO_4+ 0.0048M KOH	1.5	8.4% of NaCl	Not determined	74
Nov. 7 to 11	0.22M $C_{12}H_{22}O_{11}$+ 0.015M $MgCl_2$	0.0067M K_2HPO_4+ 0.0048M KOH	1.3	0	Not determined	64
Nov. 11 to 16	Ringer's solution[2]	0.075M K_2HPO_4	0.12	0	Not determined	52
Nov. 16 to 20	Ringer's solution[2]	0.0075M K_2HPO_4	0.6	11% of NaCl	Not determined	54
Nov. 20 to 21	Ringer's solution[2]	0.075M K_2HPO_4	−0.6[3]	Not determined	Not determined	54
Nov. 21 to 23	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	0	Not determined	Not determined	59
Nov. 23 to 25	0.3M $MgCl_2$	0.0134M K_2HPO_4+ 0.0096M KOH	1.14	Not determined	Not determined	64
Nov. 25 to 27	0.12M NaCl+ 0.015M $MgCl_2$	0.075M K_2HPO_4	0.33	0.5% of NaCl	Not determined	63
Nov. 27 to 30	0.114M KCL+ 0.015M $MgCl_2$	0.075M K_2HPO_4	0.27	1.3% of KCl	Not determined	58

Nov. 30 to Dec. 3	0.12M NaCl+ 0.015M $MgCl_2$	0.075M K_2HPO_4	−0.22[3]	2.4% of NaCl	Not determined	65
Dec. 3, 1914 to Feb. 12, 1915	0.12M NaCl+ 0.015M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.32	6.6% of NaCl	3.4% of KOH	66
Feb. 12 to 15	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0	0	0.6% of KOH	58
Feb. 15 to 18	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M NH_4OH	0	Not determined	Not determined	53
Feb. 18 to Mar. 18	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M NH_4OH	0.58	Not determined	Not determined	52
Mar. 18 to 31	0.5M $C_{12}H_{22}O_{11}$	Distilled water	19.00	10.2% of $C_{22}H_{22}O_{11}$	—	90

The experiment was carried out at room temperature, which varied between 15° and 25°.

At the end of this experiment 3.7 per cent HCl was allowed to seep through the porous cup for about 10 days under a pressure of about 20 cm. of water. It was collected, analyzed, and found to contain no Ca and 0.039 gram Mg.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] Made by adding 0.65 gram NaCl, 0.02 gram KCl, 0.025 gram $CaCl_2$, and 0.02 gram $NaHCO_3$ to 100 cc. water.

[3] Fluid passed from within the cell to the outer solution as indicated by the minus sign.

Experiment 12. December 18, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED	PERCENTAGE OF SOLUTE WHICH ENTERED CELL FROM OUTER SOLUTION	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Dec. 18 to 21, 1914	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	Not determined	0	Not determined	3
Dec. 21 to 29	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	0.86	0	Not determined	70
Dec. 29, 1914 to Jan. 4, 1915	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	1.12	0	Not determined	85
Jan. 4 to 6	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.08	3.5% of NaCl[1]	Not determined	58
Jan. 6 to 9	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.08	3.8% of NaCl[1]	0	59
Jan. 9 to 11	0.08M KCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.06	2.9% of KCl[1]	Not determined	59
Jan. 11 to 14	0.08M KCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.06	3.8% of KCl[1]	0	60
Jan. 14 to 16	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	−0.02[3]	2.1% of NaCl[1]	Not determined	60
Jan. 16 to 19	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0	3.6% of NaCl[1]	0	60
Jan. 19 to 22	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.2	0	0	65
Jan. 22 to 28	0.915M C_2H_5OH+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.54	23.5% of C_2H_5OH[2]	Not determined	65

Jan. 28 to Feb. 3	0.6M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.58	9.7% of NaCl[2]	Not determined	72
Feb. 3 to 9	1.1M C_2H_5OH+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.38	26.7% of C_2H_5OH[2]	Not determined	64
Feb. 9 to Mar. 18	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	1.08	0	Not determined	72
Mar. 18 to Apr. 7	0.5M $C_{12}H_{22}O_{11}$	Distilled water	1.2	0	—	72
Apr. 7 to June 4	0.5M $C_{12}H_{22}O_{11}$	Distilled water	3.24	0	—	100

Between 11.30 a.m., Dec. 18 and 3.30 p.m., Dec. 23, 1914 the cell used in this experiment was kept at temperatures between 29° and 35°. At 9.30 a.m., Dec. 24 the temperature had fallen to 22.5°. Between that time and 9.30 a.m. Dec. 28 it varied between 22° and 36°, after which it was kept between 28° and 33° until Feb. 25, 1915. Between this date and June 4, 1915 the cell was kept at room temperature, which varied between 14° and 26°.

[1] Quantity of solute which escaped determined by analyzing the outer solution.

[2] Quantity of solute which escaped determined by analyzing the inner solution.

[3] 0.02 cc. fluid passed from within the cup to the outer solution, as indicated by the minus sign.

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