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THE CIRCULATION OF THE BLOOD IN MAN AT HIGH ALTITUDES

I. The Pulse Rate, Arterial, Capillary, and Venous Pressures

By EDWARD C. SCHNEIDER and DWIGHT L. SISCO

(From the Department of Biology of Colorado College, Colorado Springs, Colorado)

WE purpose in a series of papers to consider circulation data obtained in Colorado Springs, altitude 6000 feet, and on Pike's Peak, altitude 14,109 feet, dealing first with the influence of low barometric pressure upon men living a comparatively inactive life muscularly and second with the influence when moderate muscular activity, and sometimes strenuous exercise such as hard mountain climbing, is added. For the prosecution of such a study Pike's Peak offers unusual advantages. It is essential that the physiological conditions, apart from the reduced atmospheric pressure, should be normal as far as possible. A cog-wheel railway ascends to the very summit of the Peak, affording easy transport for men and apparatus, which permits the elimination of muscular fatigue due to climbing. Further advantages are a substantial stone building well heated and an excellent table provided with the same variety of foods available in Colorado Springs.

In this paper appears a part of the data obtained in Colorado Springs during a period of one and a half years and in three expeditions to the summit of Pike's Peak. The first sojourn on the Peak by Havens and Schneider was for three days, October 12, 13, and 14, 1912; the second, upon which we lay most emphasis, lasted six days, May 29 through June 3, 1913, Havens, Schneider, and

Sisco being the subjects. Five men participated in the last expedition, October 23 to 27, 1913. Two, Schneider and Sisco, ascended by railway-car, while Eager, Havens, and Munro walked from Manitou to the summit. In addition to the five persons mentioned above, H. H. Robison, the resident manager of the Summit House, also served as a subject. He has now spent eighteen seasons, each of about six months, on the summit of Pike's Peak.[1]

The Frequency of the Pulse

Durig and Kolmer[2] give a critical review of the earlier studies of the pulse rate at high altitudes. They point out how greatly the heart rate varies with a variety of influences and they hold that the phenomena of circulation at high altitudes can be solved only when accidental influences are completely eliminated. Many discrepancies appear in the records of altitude studies. These are in a measure due to different conditions of living at the higher stations, such as restricted and unsatisfactory diet, poor ventilation, poor beds, and low room temperatures. Durig and Kolmer believe that circulation data obtained at low and high altitudes should be compared only in cases where the subject of observation has subsisted on a similar diet at the two altitudes and was reclining with muscles completely relaxed when under observation.

The majority of circulation records have been obtained on men who have undergone considerable physical exertion in climbing a mountain. All such records show that the heart rate increases decidedly during an ascent. Mosso[3] counted the pulse rate of five soldiers each morning before they had risen and found that the rate on the summit of Monte Rosa never sank to the minimum observed in Turin. The differences noted by him are less than those found in many other records. The acceleration at the end

[1] We wish here to offer our hearty appreciation to Mr. Leonard P. Eager, Mr. Leon C. Havens, Mr. E. Everett Munro, and Mr. H. H. Robison for serving as subjects for experimentation and for kindly help. We also take this opportunity to express our sincere thanks to Mr. C. W. Sells, President of the Manitou and Pike's Peak Railway; and to Mr. J. G. Hiestand, proprietor of the Summit House, for generous help and many facilities granted.

[2] Durig: Physiologische Ergebnisse der im Jahre 1906 durchgeführten Monte Rosa Expedition, p. 41.

[3] Mosso: Life of man on the High Alps, 1899, pp. 66 and 213.

of their sojourn was 11.7, 25, 42.4, 43.4, and 85.6 per cent respectively. Zuntz and coworkers[1] give records, from which the following figures are obtained. Using the averages of the pulse rates at Brienz (1640 feet) and Rothorn (7052 feet) and the averages at Capanna Regina Margherita (14.965 feet) on the summit of Monte Rosa, we find the percentage of acceleration was for Caspari 20.3, Loewy 28.6, Zuntz 28.9, and Kolmer 36. On the last morning of their stay the increase was 8, 20, 20.3, and 33.3 per cent. Durig and Kolmer[2] the morning following the ascent of Monte Rosa from Col d'Olen (9360 feet) found the pulse rate to be for Rainer 97, Reichel 92, Durig 87, and Kolmer 89. With one exception, a further acceleration occurred for from one to three days. The maximum increase in pulse rate was, Rainer 74, Kolmer 50, Reichel 44, and Durig 30 per cent. After this the rate retarded considerably for each man, but never sank as low as the normal for each at sea-level. In the English-American Pike's Peak Expedition[3] the pulse was not counted on the subject in bed so that these data are not wholly comparable with the above. There is, nevertheless, some similarity in events. These observations are of interest in view of our own work. The resting pulse, in the sitting position, in Douglas, Henderson, and Schneider progressively accelerated, not for three days, but for about a fortnight, after which it gradually became slower. Even after five weeks the rates were decidedly more rapid than the normals for sea-level. In Haldane the change was wholly different; the resting pulse from the first decreased until at the end of residence it was far below his sea-level rate.

The decrease in the pulse rate found in Haldane is exceptional. Mosso[4] has reported that the two keepers of the Regina Margherita Hut had the same pulse rate at the end of the season as on the plain before ascending. We have found no cases of such return to normal, nor fall below normal, among the men who spend the summer working in the hotel on the summit of Pike's

[1] Zuntz, Loewy, Müller, and Caspari: Höhenklima und Bergwanderungen, 1905, p. 337.

[2] Durig: *loc cit.*

[3] Douglas, Haldane, Henderson, and Schneider: Philosophical transactions of the royal society of London, 1913, Series B, ciii, p. 262.

[4] Mosso: *loc cit.*, p. 64.

Peak. In such persons the pulse rate, even after a residence of three and more months, has been above the normal for sea-level.

It is generally recognized that moderately high altitudes, 6000 to 9500 feet, do not in most persons cause an augmentation of the cardiac rate.

We have made a careful examination of the pulse rate in order to determine whether it is necessary for a subject to take the reclining posture with relaxed muscles in case the circulation data obtained at low and high altitudes are to be compared. For this purpose pulse counts were recorded frequently throughout the winter months in Colorado Springs and on the summit of Pike's Peak during the several expeditions.

The conditions under which we lived were quite similar at the two altitudes. The periods of observation on Pike's Peak had an atmospheric temperature variation corresponding to that of the winter in Colorado Springs. In the Summit House four rooms were placed at our disposal. The largest of these rooms, used as a laboratory, and from which each of the other rooms opened, was warmed with a stove so that the temperature was easily regulated. The beds were comfortable and clean. Our food included fresh meats, vegetables, and fruits, in fact the same variety that was to be had in Colorado Springs. The conditions of living, then, with the exception of barometric pressure, were practically the same at our two laboratories. Our physical exertion throughout was governed by the requirements of experimentation and was very similar at the two altitudes.

Pulse counts were made during the journey up the "Cog" railroad on each of us that went up by train. After equilibrium was once established the pulse rate remained constant throughout the journey and even on the summit, as we remained sitting quietly in the train after it had stopped, did not accelerate. The rates for the three of us May 29 were 60, 64, and 70. However, moderate exertion at once caused an extraordinary acceleration in pulse rate. Thus in one of us on October 12, 1912, after slowly carrying two moderately heavy suit cases 120 feet from the car to the Summit House, the pulse rate had augmented from 73 to 104. Again an hour later walking 350 paces at the rate of three miles per hour it accelerated from 80 to 120. The effect was not

so lasting as after the more vigorous exertion required to secure a similar increase at a low altitude. On the first day on Pike's Peak the return to normal after mild exertion required from two to five minutes. The acceleration for such mild work was less twenty-four hours later and marked improvement was noticed by the end of the second day.

The train on May 29 arrived at the summit at 11.25 in the morning. The members of that expedition began at once to slowly set up the apparatus, avoiding hurried movements. Throughout the day, whenever we were sitting, counts of the pulse were made. Havens' pulse continued at the tempo common for him in Colorado Springs, ranging between 60 and 78. Sisco showed more variation than ordinarily, but in the evening had counts of 65 and 67. Schneider throughout the afternoon retained on the whole his normal low altitude rate, but during the evening had an acceleration to between 85 and 90.

Schneider and Sisco each developed a headache during the first afternoon. Schneider later was mountain sick and his headache continued for several days. Sisco's headache was slight, continued throughout the first night, and wholly disappeared by noon of the thirtieth.

The pulse rates for each man of the May expedition taken in the morning before arising are given in full in the curves of Fig. I. On using the averages for the rates observed in Colorado Springs we find Havens had an acceleration of only 7.1 per cent the first morning. His heart rate was greater the second morning, had fallen the third, and reached its maximum, 21.4 per cent, the fifth or last morning. Sisco's heart had not clearly accelerated the first morning, but if the average of counts at the lower altitude is taken he showed an increase of 4.5 per cent. The rate was greater each succeeding morning and on his last had augmented 18.8 per cent. This rather moderate acceleration, which is in marked contrast with that observed by Durig and Kolmer, is accounted for by the fact that we ascended passively by train while they climbed the mountain on foot.

With Schneider the pulse changes were different; he was quite mountain sick throughout the first night. As a consequence his pulse had augmented by the first morning 46.6 per cent. Each

morning following it was found to be slower than on the day previous and by the fifth morning the rate was only 17.3 per cent above his normal.

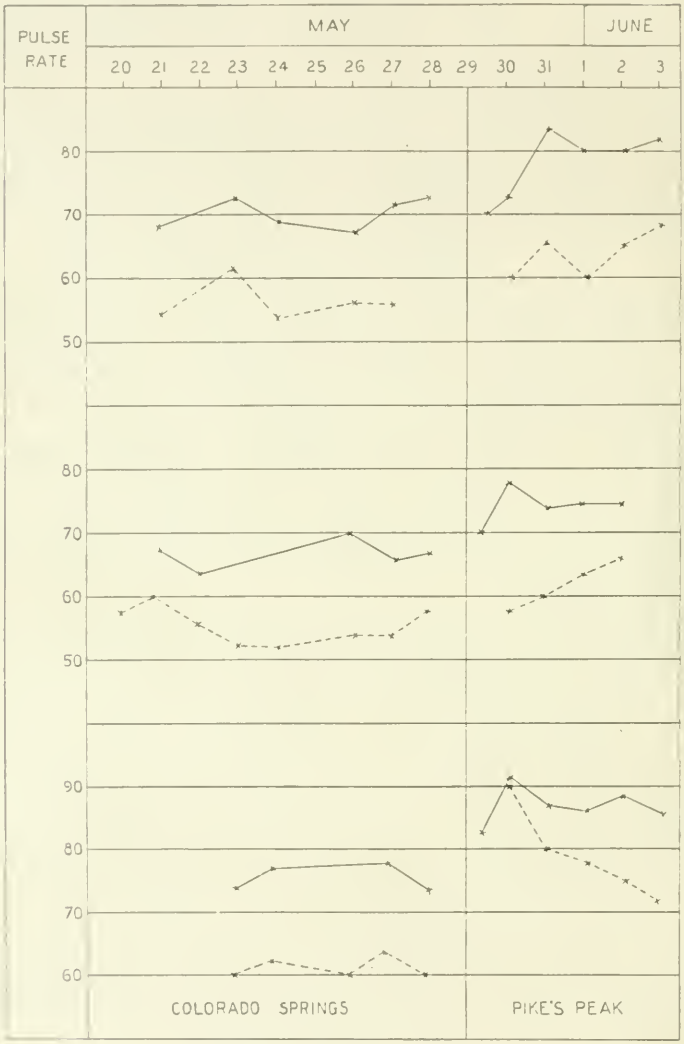

FIGURE 1

In the autumn expedition of 1913, only Schneider and Sisco of the party went up by train. Schneider, who had an average of 62 in Colorado Springs for the four mornings prior to the

ascent, had on Pike's Peak on each of the four mornings the following pulse rates: 88, 72, 74, and 76. Sisco had at the time of the ascent a slight bronchitis with no fever or other symptoms of illness. This condition was sufficient, however, to alter his reaction. Instead of a gradual daily increase in the rate, as observed in May, his heart rate had accelerated from an average of 50 in Colorado Springs to 66 the first morning, 67 the following morning, and had decreased to 62 the next two mornings.

After a residence of almost six months on the summit the pulse rate of Robison, while in bed, was exactly 64 on each of three consecutive mornings. This figure is above the rate noted in him a year earlier after his return to Colorado Springs from the summit.[1] It was then 60 for five mornings, even after he had walked to the college laboratories.

TABLE I

PULSE RATE WHILE SEATED

	Havens		Schneider		Sisco	
	Colo. Springs	Pike's Peak	Colo. Springs	Pike's Peak	Colo. Springs	Pike's Peak
Mean	70	80	76	88	68	75
Minimum	56	60	64	78	58	60
Maximum	88	92	86	100	82	86
Percentage daily variation	57.1	53.3	34.4	28.2	41.4	43.3

It was important to determine whether the heart rate of the laboratory worker, doing at the high altitude the daily routine involved in this research, was more accelerated than at the lower altitude or only proportionately augmented, when contrasted with the reclining pulse rate; and whether the accidental variations throughout the day were different than at the lower altitude. In Fig. 1 the rate of pulse found to be the average daily mean has been given with the early morning rates for three subjects.

[1] See SCHNEIDER: This journal, 1913, xxxii, p. 300.

Also in Table I are contrasted the daily variations in heart rate at the two altitudes. Changes due to exercise are not included. It is evident that the heart of each man merely took a higher daily tempo on the summit of Pike's Peak; the daily mean resting pulse curve agrees in general with the curve of the early morning rate. The tables showing arterial and venous pressures also contain pulse rates which give the details of the variation. The percentage of acceleration in what we have designated as the daily mean was for Havens 14.3, Sisco 10.3, and Schneider 15.8. The percentage increase observed throughout the day was, therefore, somewhat less for each man than his early morning acceleration of the last day. Havens' heart did not accelerate the 29th. His mean pulse rate was 73 on the 30th, had increased to 84 on the 31st, and on the following days had decreased to 80. Schneider's mean was 91 on May 30, the day he was most ill with mountain sickness. On the following days it varied between 87 and 89. Sisco also showed the highest mean rate on the 30th when it reached 79. By the following day this was down to 75 where it remained for the balance of our sojourn on the summit.

During the October, 1913, sojourn Sisco's mean pulse rate was uniformly higher, 90 the 24th and 25th and about 80 the last two days. Schneider's mean was quite similar to his of the May trip.

The fluctuation in cardiac rate due to accidental and illy defined causes was, as shown in Table I in the percentage difference between minimum and maximum, practically the same at the two altitudes. These data are also from the expedition in May, at which time physical exertion constituted only a small part of a day's routine.

The above facts and the almost constant parallel in the pulse rates at the two altitudes, when conditions are similar, justify us, we believe, in placing in contrast the altitude data of other phenomena of circulation obtained from men leading a quiet life.

Arterial Pressures

Reviews of the early literature dealing with arterial pressures have been given by one of us and Hedblom,[1] and by Durig and

[1] Schneider and Hedblom: This journal, 1908, xxiii, p. 90.

Kolmer.[1] The early records deal almost entirely with systolic pressure and fail to reveal a definite influence of high altitude in one direction; some show an increase, others no change, and still others a fall in the pressure. The same lack of uniformity appears in the data of the more recent publications.

Schneider and Hedblom reported observations made, with the Erlanger sphygmomanometer, on nine young men who climbed Pike's Peak March 27, 1907, and remained seven days on the summit. In eight of these men there was a fall in the averages of the systolic and diastolic pressures. In five the decrease was too slight to be of importance. On looking over their original notes we find that two of the men, who averaged a fall in the systolic pressure of 11 and 22 mm. respectively, had constantly a lower pressure than in Colorado Springs. The ninth man had uniformly a higher systolic pressure on the Peak and showed an average rise of 11 mm.

Durig and Kolmer employed in their expedition to Monte Rosa the Gaertner tonometer. Immediately after their arrival on the summit each man showed a marked decrease in the systolic pressure. The averages of the records of later days, however, give for three of the party an increase and for the fourth a slight fall in pressure. They conclude that in general the effect of high altitudes is to increase rather than decrease arterial pressures.

Fuchs,[2] making observations on himself on Monte Rosa with a Riva Rocci sphygmomanometer having a wide cuff, found a slight increase, average 3.22 mm., in pressure. Ward [3] about the same time, in connection with another study on the same mountain, determined his systolic pressure, which during a sojourn of seven days averaged 106 mm. This is slightly below his sea-level average of 109 mm.

The English-American Pike's Peak Expedition [4] employed a "Tycos" sphygmomanometer and concluded that while the sys-

[1] Durig: *loc cit.*, p. 69.

[2] Fuchs: Sitzungsberichten der physikalisch-medizinischen Sozietät in Erlangen, 1908, xl, p. 224.

[3] Ward: Journal of physiology, 1908, xxxvii, p. 381.

[4] Douglas, Haldane, Henderson, and Schneider: *loc cit.*, p. 262.

tolic pressures were somewhat less than under ordinary barometric pressure the differences were inconstant and so slight as to fall for the most part within the errors of observation. The diastolic pressure clearly varied only in one of the four members of that expedition and in him there was a distinct rise associated with a decided fall in the pulse pressure. These differences were, however, also inconstant.

The studies by clinical men dealing with the relation between barometric pressure and blood pressure in pulmonary tuberculosis also fail to agree. Thus Peters [1] and Bullock,[2] first working separately and later together [3] near 6000 feet, claim that altitude raises the blood pressure, while Smith [4] and Pomeroy,[5] each working separately at about the same altitude, report a fall in systolic pressure.

Realizing that many factors in activity and diet may influence arterial blood pressure, as was shown by one of us and Hedblom, we have sought in this study to make certain that the condition of each subject in these respects was comparable at both altitudes. The determinations of pressures were made with Erlanger's sphygmomanometer. To detect the systolic pressure we used the abrupt separation of the ascending and descending strokes of the pulse record as recommended by Erlanger [6] and at the same time noted the return of the pulse wave at the wrist. Determinations were always made in duplicate.

The number of determinations made has been too great to be recorded here in full. However, in order that the ordinary range of variations may be clear it has seemed best to give in detail, in tables II and IV, a part of the data obtained from each man.

The changes that occurred in the arterial pressures at the high altitude were surprisingly slight, in fact they were so slight that they fall for the most part within the errors of observation.

[1] Peters: Archives of internal medicine, 1908, ii, p. 42.

[2] Bullock: Journal of the American medical association, June, 1909, p. 23.

[3] Peters and Bullock: Archives of internal medicine, 1913, xii, p. 456.

[4] Smith: Public health reports, 1911, xxvi, No. 51.

[5] Pomeroy: Studies of cardio-vascular diseases, reprinted from the interstate medical journal, 1911, p. 105.

[6] Erlanger: This journal, 1901, vi, p. xxii.

Havens, during the sojourn in May, showed a greater range in the variation of the systolic pressure and an occasional pressure 8 mm. above his maximum at the lower altitude. These differences, however, were not found during the October stay.

TABLE II

ARTERIAL AND VENOUS PRESSURES

Havens

Date	Time	Room temperature	Pulse rate	Arterial pressures in mm. of Hg			Venous pressure in cm. of water	Place
				Systolic	Diastolic	Pulse		
Mar. 6	5 P.M.	21	66	118	90	28	18.2	Colo. Springs
11	4 "	19	78	122	78	44	11.7	
13	11.30 A.M.	21	84	118	90	28	7.4	
Apr. 29	11.30 "	22	56	115	87	28	10.2	
30	4.30 P.M.	23	72	124	85	39	11.7	
May 6	2.20 "	21	75	118	84	34	16.2	
14	3 "	22	70	124	84	40	15.0	
20	2 "	20	78	124	86	38	18.2	
21	2 "	20	70	124	84	40	11.0	
Average	—	—	**72**	**121**	**85**	**35**	**13.3**	
May 29	2.30 P.M.	21	78	110	84	26	16.9	Pike's Peak
30	10.20 A.M.	20	78	122	86	36	9.9	
30	5 P.M.	23	70	132	88	44	12.7	
31	8.20 A.M.	24	92	120	90	30	5.1	
31	2.50 P.M.	18	90	124	90	34	6.3	
June 1	8.20 A.M.	21	90	119	86	33	4.4	
1	5.30 P.M.	21	76	132	94	38	13.4	
3	8 A.M.	21	86	122	91	31	11.1	
3	11.50 "	19	80	124	93	31	9.5	
Average	—	—	**82**	**123**	**89**	**34**	**9.9**	

TABLE II (*Continued*) — *Schneider*

Mar. 5	4 P.M.	23	72	114	84	30	12.8	Colo. Springs
12	3 "	19	78	120	85	35	10.9	
Apr. 10	3.30 "	19	72	122	88	34	13.9	
25	4 "	24	77	114	82	32	16.8	
29	5 "	22	78	118	80	38	19.1	
30	3.30 "	23	77	120	82	38	14.7	
May 6	11.30 A.M.	20	68	122	90	32	15.2	
14	3.45 P.M.	22	80	120	86	34	19.2	
Average	—	—	**75**	**119**	**85**	**34**	**15.3**	
May 29	2.15 P.M.	21	82	124	86	38	19.8	Pike's Peak
29	5.30 "	21	80	128	90	38	14.0	
30	10.40 A.M.	21	88	123	88	35	13.3	
30	3 P.M.	22	100	138	94	44	20.6	
31	7.10 A.M.	27	81	130	96	34	13.7	
June 1	8.50 "	21	86	120	86	34	16.7	
1	5 P.M.	21	82	116	86	30	15.5	
2	6.30 A.M.	18	86	116	84	32	17.1	
2	12 noon	21	94	114	82	32	8.3	
3	8.40 A.M.	23	90	116	85	31	17.3	
Average	—	—	**87**	**123**	**88**	**35**	**15.6**	
			Sisco					
Mar. 5	3 P.M.	23	70	114	84	30	6.2	Colo. Springs
Apr. 10	4 "	19	74	122	85	37	7.2	
25	4.30 "	25	68	112	84	28	9.0	
29	5.20 "	22	60	120	80	40	8.5	
30	4 "	23	78	114	86	28	10.4	
May 6	4 "	21	66	114	88	26	8.6	
8	4.15 "	21	62	118	84	34	9.8	
22	11 A.M.	19	70	126	84	42	8.2	
Average	—	—	**69**	**118**	**84**	**33**	**8.5**	

TABLE II (*Continued*)

Sisco (*Continued*)

Date	Time	Room temperature	Pulse rate	Arterial pressures in mm. of Hg			Venous pressure in cm. of water	Place
				Systolic	Diastolic	Pulse		
May 29	2 P.M.	21	86	—	—	—	11.4	Pike's Peak
29	4.30 "	21	70	120	86	34	11.6	
30	10 A.M.	20	70	110	84	26	− 0.6	
30	3.30 P.M.	20	80	116	85	31	1.5	
31	9 A.M.	23	80	114	83	31	2.1	
May 31	4 P.M.	20	72	120	88	32	− 0.2	Pike's Peak
June 1	8 A.M.	21	82	118	84	34	− 0.7	
1	5.20 P.M.	21	72	120	88	32	2.1	
2	6.45 A.M.	21	76	116	80	36	0.9	
2	2 P.M.	21	80	112	78	34	1.8	
Average			**77**	**116**	**84**	**32**	**3.0** or (**0.9**)	
			Eager					
Oct. 13	3.40 P.M.	20	81	116	84	32	14.5	Colo. Springs
17	11.40 A.M.	21	72	128	88	40	15.0	
18	3.30 P.M.	20	82	108	82	26	10.4	
Nov. 20	9.45 A.M.	21	84	126	84	42	11.0	
Dec. 2	12.25 P.M.	17	82	114	84	30	11.9	
5	10.55 A.M.	19	84	126	82	44	12.6	
15	1.55 P.M.	19	86	126	88	38	15.0	
Average	—	—	**82**	**121**	**85**	**36**	**12.9**	
Oct. 24	8 P.M.	23	96	118	84	34	3.6	Pike's Peak
25	9 A.M.	20	100	106	78	28	8.2	
26	9 "	23	96	114	82	32	9.2	
27	10 "	16	87	116	87	29	1.5	
Average	—	—	**95**	**114**	**83**	**31**	**5.6**	

TABLE II (*Continued*)

Munro

Oct. 7	11.25 A.M.	19	84	122	90	32	9.7	Colo. Springs
10	4.35 P.M.	20	74	116	78	38	15.0	
20	2.30 "	16	78	116	84	32	15.4	
Nov. 20	10.10 A.M.	21	72	116	89	32	11.7	
26	10.45 "	19	72	118	82	36	14.8	
27	11.45 "	21	82	118	84	34	15.0	
Average	—	—	**77**	**118**	**85**	**34**	**13.6**	
Oct. 24	8.30 P.M.	23	88	118	86	32	4.4	Pike's Peak
25	8.50 A.M.	20	100	114	82	32	6.9	
26	8.50 "	24	95	122	89	33	2.6	
26	9.30 "	24	94	118	82	36	− 1.4	
27	6.45 "	14	84	117	89	28	− 4.2	
Average	—	—	**92**	**118**	**86**	**32**	**1.7**	

Schneider was, it will be recalled, mountain sick during the first days of the spring expedition and it was during those days, the first three, that the arterial pressures averaged unusually high: systolic 129, diastolic 91, and the pulse pressure 38. The averages for the three days show the following increase over the Colorado Springs averages: the systolic 10 mm., diastolic 6 mm., and pulse pressure 4 mm. However, on the morning of the fourth day there was a marked fall in his pressure and throughout the remainder of the stay the systolic and pulse pressures were each slightly below his Colorado Springs averages. During the October trip Schneider was ill only the first night and with this better health there failed to develop the early period of high pressure; his arterial pressures then conformed throughout the stay with those found to occur at the lower altitude. Observations made on Schneider with a "Tycos" sphygmomanometer by the English-American Pike's Peak Expedition in 1911 show a 5 mm. fall in his average systolic pressure and 7 mm. decrease in the pulse pressure. With him then, when in health, it seems established that at the altitude

of 14,000 feet the arterial pressures are practically unchanged or at the best only slightly lowered.

Eager, who was one of the three to walk up the Peak, showed on the mountain an average decrease of 7 mm. in the systolic and 5 mm. in the pulse pressure but no change in the diastolic pressure.

In Munro and Sisco (during his first trip) we could find no constant change in any of the three pressures while they were on Pike's Peak. Sisco had in the October expedition a slight decrease in the systolic and pulse pressures.

TABLE III

AVERAGE ARTERIAL AND VENOUS PRESSURES FOR THE FOUR PERIODS

Person	Period	Place	Pulse rate	Arterial pressures in mm. of Hg			Venous pressure in cm. of water
				Systolic	Diastolic	Pulse	
Havens	Feb. 1–May 21	Colo. Springs	67	120	86	34	12.8
"	May 29–June 3	Pike's Peak	83	122	89	33	9.9
"	Oct.–Dec.	Colo. Springs	74	126	90	36	12.4
"	Oct. 23–27	Pike's Peak	87	125	92	33	9.8
Schneider	Feb. 1–May 21	Colo. Springs	73	118	84	34	16.2
"	May 29–31	Pike's Peak	88	129	91	38	15.7
"	June 1–3	" "	86	116	85	31	
"	Oct.–Dec.	Colo. Springs	76	121	85	36	15.4
"	Oct. 23–27	Pike's Peak	86	118	85	34	14.5
Sisco	Feb. 1–May 21	Colo. Springs	66	118	85	33	8.9
"	May 29–June 3	Pike's Peak	75	117	84	33	2.5
"	Oct.–Dec.	Colo. Springs	70	119	86	33	7.2
"	Oct. 23–27	Pike's Peak	90	115	85	30	1.4

In Table III are given the averages of all normal arterial pressures obtained from Havens, Schneider, and Sisco for the two periods of observation in Colorado Springs and for the last

two expeditions up the Peak. There is a remarkable likeness in the data for the two periods at each altitude. What differences occur are on the whole slight and, appearing as they do in the readings at both altitudes, suggest that these are incidental and not the effect of altitude.

Since the two sets of pressure determinations for Havens, Sisco, and Schneider are in such complete agreement for the two sojourns on Pike's Peak, we feel we are justified in concluding that we have succeeded in eliminating disturbing factors and that the conditions of life were, excepting barometric pressure, practically the same at the low and high altitudes.

The observations on Robison, given in Table IV, are of more than passing interest in that he spends six months of each year on the summit. Schneider[1] has recently shown that during a period of six years in which Robison has been under observation his arterial pressure has remained normal. A few of our determinations were made last spring before Robison ascended the Peak. When these are contrasted with the pressures obtained on the mountain the high altitude readings show an average fall of 6 mm. in each of the systolic and pulse pressures, but no change in his diastolic pressure. The decrease observed in the pulse pressure is in harmony with our recoil curve records in which we find the recoil for him less than that of the other subjects of our investigation.

These data from Robison are in complete agreement with those obtained on him by Schneider and Hedblom[2] in 1907. They record a series of eighteen observations in which his systolic pressure had on Pike's Peak an average decrease of 7 mm. and pulse pressure 5 mm.

There are among the records of this laboratory arterial pressure determinations on five employees of the hotel who have resided from ten days to several months on the summit of the Peak. For these men the systolic pressure ranges from 104 to 134, the diastolic from 75 to 90, and the pulse pressure from 26 to 50 mm.

Data accumulated during the past five years from determina-

[1] Schneider: This journal, 1913, xxxii, p. 299.

[2] Schneider and Hedblom: *loc. cit.*, p. 101, Table VI.

tions on ninety young men of college age show that for about 80 per cent the average systolic pressure at an altitude of 6000 feet is less than 120 mm. Our experience leads to the opinion that at an altitude of 6000 feet normal healthy young men show the same range and distribution of pressures as young men do at sea-level.

TABLE IV

ARTERIAL AND VENOUS PRESSURES. (ROBISON)

Date	Time	Room temperature	Pulse rate	Arterial pressures in mm. of Hg			Venous pressure in cm. of water	Place
				Systolic	Diastolic	Pulse		
Jan. 28	7.30 A.M.	—	76	116	84	32	—	Colo. Springs
30	8.00 "	—	70	114	80	34	—	
May 1	7.45 "	21	66	114	84	30	13.3	
1	9.35 "	21	66	—	—	—	15.7	
Nov. 23	11.45 "	19	77	118	82	36	11.8	
Average	—	—	**71**	**116**	**83**	**33**	**13.6**	
May 31	10.00 A.M.	18	90	110	84	26	13.9	Pike's Peak
31	5.30 P.M.	21	84	106	80	26	7.9	
June 1	9.00 A.M.	21	86	110	84	26	12.5	
1	5.40 P.M.	21	81	114	84	30	4.0	
3	9.10 A.M.	22	96	112	84	28	11.2	
Average	—	—	**87**	**110**	**83**	**27**	**9.9**	

Of special interest are the data on pulse pressure, the difference between systolic and diastolic arterial pressure, which is generally taken as evidence of the volume of the systolic discharge or the size of the heart strokes. Our own data and those of Douglas, Haldane, Henderson, and Schneider indicate that in the majority of persons the pulse pressure does not definitely alter at high altitudes and in those in which a change occurs there will be a slight decrease in the output of the heart.

We conclude, from our experience in these several expeditions and from the data accumulated in this laboratory during the past six years, that for many, and very likely the majority of healthy men, resi lence at very high altitudes does not influence the arterial pressures. In a certain, but as yet undetermined, percentage of men it will cause a demonstrable fall in the systolic and pulse pressures, and in very exceptional cases will bring about a marked rise in the arterial pressures. This rise was observed by Schneider and Hedblom in one man.

Venous Pressure

For the determination of venous pressure we have constructed an instrument which is a modification of the one described by Hooker and Eyster.[1] Experience with two of the Hooker-Eyster instruments made here demonstrated that the rectangular form of the box is a difficult one over which to fit the rubber dam by means of the metal collar. Only after repeated trials was it possible to secure an air-tight chamber and this would hold only a few days; in that time the rubber skirt would be cut at the angles by the collar.

Our instrument[2] is a cylindrical glass box 5 cm. in diameter and 1.8 cm. in height. The top is a thin watch-glass cemented to a narrow thin metal band which in turn is cemented to the glass cylinder. The metal band has a tube entering it which connects the chamber with a manometer and also has attachments for the binding tapes which hold the instrument in place on the arm. Over the lower end of the cylinder a thin metal band is cemented and a metal collar 4 mm. wide holds the rubber sheet in place. The rubber sheet is easily placed in position, and should leaks occur around the band these are readily closed with rubber cement or balsam, after which the instrument is ready for constant use for several months or until the rubber deteriorates.

For use a rectangular opening is cut in the rubber covering of the bottom. The box, after the plan employed by Hooker and

[1] Hooker and Eyster: Johns Hopkins hospital bulletin, 1908, xix, p. 274.

[2] We are indebted to Mr. E. E. Taliaferro for suggestions and for making our instrument.

Eyster, is connected with a water manometer and rubber pressure bulb so that the pressure is transmitted directly to the box and manometer. The instrument can readily be placed on the arm or hand in such a manner that it does not exert pressure on the veins.

Our determinations of the venous pressure have all been made with the above instrument on the back of the hand or wrist. Pressure was applied rapidly until the vein collapsed and the manometer was then read by an assistant or the subject of experimentation. Pressure was next lowered and again applied and this repeated until ten or fifteen readings had been made. The average of these readings was then taken and a correction made by deducting the difference in level between the vein and the right auricle of the heart. This level of the heart was considered to be the mid point at the anterior-posterior diameter of the body at the tip of the sternum, the subcostal angle, the point arbitrarily chosen by v. Rechlinghausen.[1]

We have not to date determined the venous pressure of many different individuals. A limited number of observations on seventeen men indicate that the pressure range at 6000 feet is about the same — 2 to 16 cm. of water — as Hooker[2] found in Baltimore. An occasional reading, for which an explanation was not apparent, has been as high as 20 cm.; but in not a single case has this high pressure been found to be the normal condition for an individual. Since the number of individuals examined has been so small and on several of the men only one and two determinations have been made, we give only a tentative estimate of the average value of the venous pressure in Colorado Springs, which, with our instrument, we find to lie between 10 and 11 cm. of water. Hooker gives 9 cm. as the average value near sea-level. This difference in view of experience on Pike's Peak cannot be attributed to altitude influence but rather to instrumental and technic differences.

Venous pressures have not been studied heretofore at extremely high altitudes. Oliver[3] made observations on two subjects, aged

[1] v. Rechlinghausen: Archiv für experimentelle Pathologie und Pharmacologie, 1906, lv, p. 463.

[2] Hooker: This journal, 1911, xxviii, p. 235.

[3] Oliver: Blood and blood pressure, 1901, p. 204.

23 and 57, on a visit to Arosa, altitude 5800 feet, finding their venous pressures high. He fails to give data. Sewall [1] finds evidence of overstrain of the right side of the heart in certain cases newly arrived in Denver (5300 feet) from lower levels, from which he concludes that the venous pressure at least in such cases is abnormally high. However, his determinations on healthy men with an instrument designed by himself [2] show an average pressure of about 4 mm. Hg. This is below the average for near sea-level as given by Hooker.

A part of the data on venous pressure secured in our expeditions to Pike's Peak appears in tables II, III, and IV. They show a decided fall in this pressure for Eager, Havens, Munro, and Sisco, but no change for Schneider. The percentage of the fall in the averages of the venous pressure on Pike's Peak was for Munro 87, Sisco 72, Eager 57, and Havens 25. Individual differences were in evidence. Sisco during the first half day on the summit had on both sojourns a high venous pressure; in the first instance it was above that of any determination made in Colorado Springs. During the first night on the summit his pressure lowered and frequently thereafter was slightly negative; at one time it was 3 cm. below atmospheric pressure. Munro likewise often showed a negative venous pressure; once this was fully 4 cm. The highest pressure obtained on Havens occurred the first half day of the first expedition. His venous pressure was more variable than that of any other of our subjects. He generally had a higher pressure in the morning than in the afternoon.

The averages of the venous pressures for Havens, Schneider, and Sisco for the two expeditions appear in Table III. The changes observed the second trip almost duplicate those of the spring expedition.

The observations on Robison (see Table IV), who spends six months of each year on the summit of Pike's Peak, show his venous pressure to fluctuate as did Havens'. It should be noted that this pressure for him was always higher in the morning than in

[1] Sewall: Transactions of the American climatological association, 1906, xxii, p. 122.

[2] Sewall: Journal of the American medical association, 1906, xlvii, p. 1279.

the afternoon; in fact in the morning it was as high as in Colorado Springs. Only a small number of determinations have been made on Robison in Colorado Springs, but they average more than his average pressure on Pike's Peak. This average shows a fall of 27 per cent on the Peak.

While on Pike's Peak the venous pulse was noted on three of us in the recumbent position. Since we were not equipped for recording this pulse we were compelled to note only the visible changes. In Havens and Schneider the external jugular veins appeared as full and the pulse as pronounced as at the lower altitude. With Sisco in the reclining position at a time when the venous pressure was -0.8 the jugulars were visible and the pulse fairly distinct. A rock weighing about 25 lbs., then placed on Sisco's abdomen, raised his venous pressure to 1.6 cm. and somewhat accentuated the venous pulse. This observation demonstrated that the splanchnic reservoir at the high altitude did not contain more blood than at the altitude of Colorado Springs, since there we had been able to increase the venous pressure a little over 4 cm. with a similar weight.

The venous pressure, as shown by Krogh [1] and by Henderson and Barringer [2] determines the extent of filling of the right ventricle and is thus the principal factor controlling the volume of the arterial blood stream. It may be questioned whether men with as low a venous pressure as was sometimes found in Munro and Sisco on Pike's Peak do not have a greatly reduced systolic discharge. A study of sphygmograms and of our recoil-board curves do not indicate such a reduction. Furthermore, Henderson and Barringer [3] have shown that a venous pressure above that which they designate as the critical venous pressure will not increase the diastolic filling of the ventricle; i.e., the heart will begin to beat with an efficiency which is maximal for a given rate when the critical venous pressure is reached. They find that the critical venous pressure necessary to distend the dog's right ventricle as rapidly as it relaxes is not more than 50 mm. of saline. They deem it probable that in the adult human heart the larger

[1] Krogh: Skandinavisches Archiv für Physiologie, 1912, xxvii, p. 227.
[2] Henderson and Barringer: This journal, 1913, xxxi, p. 288.
[3] Henderson and Barringer: *loc. cit.*, p. 352.

size involves a somewhat greater critical pressure, but consider that the negative pressure of the chest would provide an effective pressure of at least the critical value. The normal intrathoracic negative pressure in expiration at sea-level is at least −60 mm. of water and in inspiration −100 mm. or more. The thoracic negative pressure must average somewhat higher at extreme altitudes in that the depth of inspiration is increased, it having been shown by Douglas, Haldane Henderson, and Schneider [1] that the rate and depth of breathing on Pike's Peak is such as to cause the inhalation of 30 per cent more air per minute. Since the rate of respiration is only slightly or not at all increased it follows that the depth will increase and with it the negative pressure. Furthermore, the lessened atmospheric pressure very likely leads to greater negative pressure even during expiration. Whatever the average negative intrathoracic pressure may be on Pike's Peak it is possible that there may occur periods of such low venous pressure in some men, examples Munro and Sisco, that for a time this pressure is below the critical venous pressure. This condition, however, is only occasional in men so far examined. In the majority of men the venous pressure is always positive and even in those in which it is at times negative it is the greater part of the day positive; hence it follows that the venous supply and pressure are generally sufficient at the high altitude to give a maximal efficiency of heart stroke.

The cause of the fall in the venous blood pressure at the high altitude appears to be the increased rate of the heart beat, which permits an increased outflow from the large veins. The probable decrease in intrathoracic pressure may also be a supplementing factor. That diminution of ventricular output was not responsible for the fall in venous pressure was evidenced by our recoil-board records, in which the height of the curves were practically the same at both altitudes; that it was not due to a stagnation of the blood in the splanchnic reservoir was shown by pressing the blood from this area into the systemic or external circulation by means of weights placed on the abdomen of men in the reclining position, since in each man this increase in pressure was no greater than that secured in Colorado Springs; that it was not caused

[1] Douglas, Haldane, Henderson, and Schneider: *loc. cit.*, p. 217.

by a contraction of the arterioles was proven by the fact that the arterial pressures were not increased, also by the fact that the capillary pressures were practically unaltered.

Capillary Pressures

For these determinations we employed a Lombard pressure chamber which we had constructed in accordance with his specifications.[1] With this instrument the skin is wet with glycerine and the smaller blood vessels are viewed with a microscope as the pressure is applied. The obliteration of the blood vessel is observed rather than the blanching of the skin as in older methods. We eliminated the Wolf-bottle suggested by Lombard for applying pressure and employed instead the same device that was used with the venous pressure apparatus. A Nernst lamp was the source of illumination in Colorado Springs while on the summit of Pike's Peak it became necessary to use sunlight which was reflected onto the microscopic field by means of a mirror.

Unfortunately adequate light could not be obtained in the laboratory on the summit so that the capillary readings on the mountain had to be made out of doors. This made it impossible to regulate the temperature so that conditions would be similar to those experienced at the lower altitude. Sheltered places were found near the Summit House, but even then the temperatures were much below those in the laboratory. Because of this and frequent cloudy weather the number of determinations on the Peak was much limited.

The capillary determinations which have been corrected for the intercostal angle appear in Table V. The most compressible capillaries disappeared at fully as low pressures on Pike's Peak as in Colorado Springs. To obliterate the majority of capillaries required for Havens and Sisco a slightly greater pressure on the Peak than was usually necessary in Colorado Springs, but was not above pressures often found at the lower altitude. For Schneider in one trial the majority of capillaries disappeared at the same pressure as the average in Colorado Springs, but in the second determination on another day it was above this average. The

[1] Lombard: This journal, 1912, xxix, p. 347.

most resistant capillaries were obliterated by pressures of 67 mm. Hg and under. Our readings both in Colorado Springs and on Pike's Peak fall within the ranges of variation established by Lombard for near a sea-level altitude. He found the pressures

TABLE V

CAPILLARY PRESSURES

Subject	Place	Reading	Pressures in mm. of Hg		
			First capillaries	Majority of capillaries	Last capillary
Havens	Colo. Springs	Average	21	37	56
"	" "	Lowest	14	30	44
"	" "	Highest	27	52	63
"	Pike's Peak	May 31	21	47	—
"	" "	31	21	42	—
"	" "	June 2	12	44	67
Schneider	Colo. Springs	Average	24	39	54
"	" "	Lowest	18	33	48
"	" "	Highest	31	43	67
"	Pike's Peak	May 31	17	37	—
"	" "	June 2	19	50	66
Sisco	Colo. Springs	Average	26	42	58
"	" "	Lowest	16	30	54
"	" "	Highest	38	53	61
"	Pike's Peak	May 31	27	48	—
"	" "	June 2	16	46	60

in millimeters of mercury to vary for the most compressible capillaries between 15 and 25, the average capillaries 35 to 45, and the most resisting capillaries 60 to 70.[1]

[1] LOMBARD: *loc. cit.*, p. 362.

According to Hough,[1] also Oliver,[2] low temperatures (from 5° to 15° C.) increase capillary pressure by causing a venous constriction. Our determinations on Pike's Peak were out of doors under a temperature of 10° while those in Colorado Springs were at temperatures varying from 17° to 22° C. This, applied to our data, suggests that the capillary pressure on Pike's Peak at ordinary room temperatures would be at least the same or slightly less than in Colorado Springs. Bayliss and Starling[3] have shown, when the capillary pressure cannot be measured directly, that simultaneous determinations of arterial and venous pressures will give reliable information as to the variation of capillary pressure; that in cases in which one of these falls while the other remains constant the capillary pressure must be diminished, and in cases where arterial and venous pressures rise or fall together the capillary pressure rises or falls with them. From our arterial and venous determinations we are, therefore, warranted in concluding that the capillary pressure is decreased in some men and unaltered in others by residence at high altitudes.

It has frequently been claimed that bleeding from nose, lips, gums, lungs, or stomach is a common experience at high altitudes and this has been attributed to increased capillary pressure. Among the thousands of people that one of us has seen ascend Pike's Peak there have been very few cases of hemorrhages and these of the nose only. Such cases are so rare that doubt would be thrown on the usual explanation even in the absence of the positive proof that capillary pressure is not increased.

The Mass-movement of the Blood Shown by a Recoil Curve

The device employed has been described by Yandell Henderson[4] and was used by the English-American Pike's Peak Expedition.[5] A plank, or recoil board, was supported upon rubber stoppers, a large and a medium size stopper placed one upon the

[1] Hough: This journal, 1900, iii, p. xii.
[2] Oliver: Studies in blood pressure, 1908, p. 77.
[3] Bayliss and Starling: Journal of physiology, 1894, xvi, p. 159.
[4] Yandell Henderson: This journal, 1905, xiv, p. 290.
[5] Douglas, Haldane, Henderson, and Schneider: *loc. cit.*, p. 267.

other under each corner. By means of a small upright of wood a stiff wire hook was connected with a lever in such a manner that the movements of the board were magnified sixty times and recorded upon a smoked drum. The amplitude of the graphic record affords an index of the volume of blood propelled by each heart stroke in relation to the body weight.

Since our curves are similar to those obtained by Douglas, Haldane, Henderson, and Schneider, and our results in complete agreement with their work, they are here omitted. The amplitude of the curve varied on the whole with the pulse pressure, and the variations were practically the same at the low and high altitudes. The amplitude of the curve for Robison was uniformly less than that of the other subjects. Unfortunately no records are available for him at the lower altitude. It is, however, of interest to find his pulse pressure also less at the high altitude. When the heart rate reached a hundred and over there was as a rule a reduction in the amplitude of the curve.

It is evident from our observations on the pulse, the recoil curve, and the various pressures that in the men examined the conditions were favorable for an increase in the rate of blood flow while they were on the summit of Pike's Peak. The amplitude of the heart beat, as shown by the pulse pressure and recoil curve, was practically unaltered in four of the six men examined. In the two exceptions the pulse pressure showed a diminution of 14 and 18 per cent. Each subject examined had a marked acceleration, 10 to 21 per cent, in heart rate on the Peak, the greatest occurring in the case of greatest fall in pulse pressure. If the pulse rate be multiplied by the pulse pressure and the product be taken as a relative measure of the volume of the blood stream per minute, for each subject a marked increase in the circulation rate is indicated. The output of the heart per minute being increased without a corresponding rise in the arterial systolic pressure, a readjustment in the peripheral resistance, i.e., the arterioles, must occur so as to permit a more abundant flow of blood through the capillaries. The arterial systolic pressure was unchanged in four of our subjects and lowered in two, thus indicating that such readjustment did occur. Furthermore, in all but one

man a lowering of the venous pressure was found, thus giving another factor favoring an increase in the rate of circulation. With the physiologic resistance in the circuit decreased, or even remaining unaltered, a fall in pressure at the outflow end can only give an increase in the velocity of flow.

Summary

1. The pulse rate does not accelerate immediately on arrival at an altitude of 14,109 feet, but requires several days to reach its maximum. Very moderate exertion at first brings on an extraordinary, but brief, acceleration, an effect which is less marked by the second or third day. In mountain sickness the rate augments rapidly, to retard on recovery to high altitude normal. The daily mean pulse rate for a subject in the sitting posture while rapid, shows approximately the same proportionate increase as the early morning rate does when compared with rates at the lower altitude. The daily fluctuation of heart rate due to accidental and illy defined causes was found to have approximately the same percentage range at low and high altutides.

2. In the majority of healthy men at high altitudes the arterial pressures are unchanged. Some men may experience a moderate decrease in the systolic and pulse pressure, the diastolic remaining most constant. In about 80 per cent of young men of college age at the altitude of 6000 feet the normal systolic pressure is under 120 mm. Hg.

3. Venous pressure determinations, made with a new form of instrument, show the pressure at an altitude of 6000 feet to vary from 2 to 16 cm. of water, the range of variation recorded by Hooker for sea-level. In five out of six subjects on Pike's Peak the venous pressure was lowered from 25 to 87 per cent. In two men it was at times slightly negative.

4. The capillary pressure was not clearly altered by reduced barometric pressure.

5. A study of the mass-movement of the blood by the recoil-board method and observations on the pulse pressure show the volume of ventricular output per heart stroke to be the same for four of the men at both altitudes and to be clearly reduced for one man at the high altitude.

6. In consequence of the increased pulse rate and fall in venous pressure and the unchanged or only slightly lowered arterial pressure, conditions of the vascular system favor an increased rate of blood flow on Pike's Peak. An adaptive response in the mechanism regulating the peripheral resistance is predicated.

THE CIRCULATION OF THE BLOOD IN MAN AT HIGH ALTITUDES

II. The Rate of Blood Flow and the Influence of Oxygen on the Pulse Rate and Blood Flow

By EDWARD C. SCHNEIDER and DWIGHT L. SISCO

[*From the Department of Biology of Colorado College, Colorado Springs, Colorado.*]

THE data here presented have been obtained from the same men who served as subjects in our study of the pulse rate, arterial, capillary, and venous pressures.[1] Three men participated in the first expedition (May 29–June 3), while these and two additional men were members of the last expedition (October 23–27, 1913) to the summit of Pike's Peak. A sixth subject was the resident manager of the Summit House.

Blood Flow in the Hands

We have sought throughout our study to determine how and to what extent the circulation rate is altered by residence at very high altitudes. Naturally the best way to settle the question would be a direct determination of the volume of the systolic discharge per minute into the aorta. Unfortunately no simple method has as yet been devised that will even indirectly give satisfactory data as to the volume of the heart output in man. Since the oxygen-want at high altitude stimulates the blood-forming centers to increase the percentage and the total amount of hemoglobin in the blood, the lungs to actively secrete oxygen, and the respiratory mechanism to a greater ventilation of the lungs, all so that the tissues may be more adequately supplied with oxygen; it has seemed almost certain that the rate of blood flow must also be increased for the same purpose. A more rapid

[1] See this journal, 1914, xxxiv, p. 1.

rate of blood flow could raise to a limited extent the oxygen pressure in the blood passing through the tissues and so ensure better oxidation within the tissues. The problem then to be solved is not so much the amount of the systolic discharge but rather the relative rate of the blood flow through any organ or tissue at low and high altitudes. We have, therefore, made determinations of the amount of blood flow in the hands.

The determinations of the blood flow were made by Stewart's[1] method. For this purpose we constructed and equipped two hand-calorimeters according to his specifications. The method determines the amount of heat given off by the resting hand in a given time, and indirectly the temperatures of the arterial and venous blood in the part. With these data it is possible to calculate how much blood has passed through the hand in order that it might give off the determined amount of heat. The method undoubtedly gives values of blood flow somewhat less than the amount actually passing through the hand. It is, nevertheless, a simple method which measures the rate with a considerable degree of accuracy and has served well in our problem in which evidence as to change in the rate of the flow was demanded.

Experience early showed us that muscular activity of any kind accelerates the blood flow. Hence in our study we have made determinations after the subject had been quiet for several hours and as often as possible on days in which he had not indulged in strenuous work or exercise. The conditions of the subject and of experimentation correspond at the two altitudes. It was impossible to have an absolutely uniform room temperature throughout. However, Hewlett[2] has shown that room temperatures between 18 and 25.5° C. do not materially alter the blood flow in the arm. With but one exception our room temperatures fell within this range.

In all experiments the subject was required to immerse the hands for at least ten minutes in a bath of the same temperature as the water of the calorimeters in order that equilibrium in the circulation should be attained. Then while sitting comfortably he held his hands in the calorimeters for from 10 to 30 minutes;

[1] Stewart: Heart, 1911, iii, p. 33.

[2] Hewlett: Heart, 1910, ii, p. 230.

the water was stirred almost constantly and the temperature read at intervals of two minutes.

While our data have been obtained from six men, three (Havens, Schneider, and Sisco) were studied more thoroughly at both altitudes than the others. In each of the six subjects the amount of the blood flow through 100 c.c. of hand volume was greater on the summit of Pike's Peak than in Colorado Springs. Table I contains these data. Since there are individual differences requiring explanation each subject will be discussed separately.

For Havens the average increase in the blood flow on Pike's Peak was for the right hand 65 and the left hand 70 per cent. This subject during the spring months was in training for the two mile run and consequently exercised daily. The determinations in Colorado Springs on March 20, April 1 and 15, were made during the afternoons and were preceded by a morning workout on the track. During the summer and autumn he had taken almost no exercise and spent much of each day in laboratory work. It may be only a coincident and not the result of the physical condition of the subject that his blood flow in the hands averaged in Colorado Springs about 50 per cent less in the autumn when out of training than during the spring months of vigorous exercise. His average amount of blood flow in Colorado Springs during the spring months was 8.6 grams per minute in 100 c.c. of hand and for the fall 4.2 grams. A further point indicating the value of physical fitness is found in the smaller increase in the blood flow during the expedition to the summit in May. At that time the percentage increase was for the right hand 24 and the left hand 29, much below the average of increase for the two expeditions. It should be borne in mind that he walked up the mountain in October and this may account for the constantly higher rate of flow during the autumn expedition.

The variations in Havens' records while on Pike's Peak may be explained in part. The high rates of June 1, October 23 and 25, were associated with an accelerated pulse rate and on each occasion the pulse pressure was 36 mm., which was a little above his average of 33 mm.

Schneider had about the same rate of blood flow during both sojourns on the summit with an average increase of 76 per cent

TABLE 1

Results of Experiments on the Blood Flow in the Hands

Havens

Date	Place	Time	Pulse Rate	Temperature in		Volume of hand in c.c.		Blood flow in grams per minute		Blood flow in grams per 100 c.c. of hand per minute	
				Rectum	Room	Right	Left	Right	Left	Right	Left
Mar. 20	Colo. Springs	3.15 P.M.	80	37.50	18.4	432	429	30.9	28.7	7.2	6.7
Apr. 1	" "	3.30 "	80	37.84	21.9	451	451	48.2	47.1	10.7	10.4
15	" "	2.35 "	78	38.17	20.4	412	437	35.4	37.8	8.6	8.7
May 13	" "	10.55 A.M.	68	37.44	21.5	430	423	36.2	36.9	8.0	8.7
Nov. 19	" "	4.30 P.M.	80	37.33	20.0	452	431	22.0	23.9	5.2	5.5
26	" "	4.30 "	68	38.05	19.0	423	405	19.3	11.8	4.5	2.9
Dec. 18	" "	4.20 "	72	37.54	20.3	423	433	14.2	15.4	3.4	3.6
										6.8	6.6
May 29	Pike's Peak	9.15 P.M.	75	36.67	24.3	402	420	41.8	44.7	10.4	10.6
30	" "	12.20 "	74	37.50	18.0	410	429	38.6	41.1	9.4	9.6
June 1	" "	12.40 "	88	37.86	21.5	400	384	52.9	53.4	13.2	13.9

2	" "	11.10 A.M.	83	38.00	21.1	423	395	41.5	40.7	9.8	10.3
Oct. 23	" "	7.00 P.M.	100	37.33	16.0	423	412	52.3	46.6	12.4	11.3
25	" "	8.00 A.M.	92	37.44	19.0	419	434	49.8	51.1	11.8	11.5
										11.2	**11.2**
				SCHNEIDER							
Mar. 20	Colo. Springs	4.30 P.M.	76	37.56	19.1	396	401	23.2	27.4	5.9	6.8
Apr. 1	" "	11.20 A.M.	76	37.22	21.4	430	—	22.8	—	5.3	—
15	" "	10.55 "	84	37.67	20.5	388	374	26.7	24.4	6.9	6.6
May 13	" "	2.30 P.M.	82	37.56	21.8	398	388	21.1	23.8	5.3	6.1
Nov. 14	" "	9.00 "	80	37.11	21.0	420	395	41.3	31.6	9.5	7.3
Nov. 20	" "	11.30 A.M.	72	36.84	20.0	409	398	16.3	16.4	3.8	4.1
Dec. 18	" "	3.20 P.M.	84	37.17	20.4	403	—	18.3	—	4.5	—
										5.9	**6.2**
May 29	Pike's Peak	8.30 P.M.	90	36.62	23.8	405	388	39.3	33.1	9.7	8.5
30	" "	2.25 "	100	37.56	20.5	398	383	43.1	43.4	10.8	11.3
June 1	" "	12.05 "	92	37.33	20.6	404	395	35.0	34.8	8.7	8.8
2	" "	11.40 A.M.	94	37.56	20.6	405	402	47.7	38.6	11.8	9.6
Oct. 24	" "	12.30 P.M.	102	37.44	21.0	404	398	41.6	40.0	10.3	10.0
25	" "	7.15 "	92	37.22	20.0	424	407	46.3	35.3	11.0	8.7
										10.4	**9.5**

TABLE 1 (*Continued*)

Sisco

Date	Place	Time	Pulse Rate	Temperature in		Volume of hand in c.c.		Blood flow in grams per minute		Blood flow in grams per 100 c.c. of hand per minute	
				Rectum	Room	Right	Left	Right	Left	Right	Left
Mar. 21	Colo. Springs	11.00 A.M.	60	37.12	19.7	417	419	20.6	23.5	4.9	5.6
Apr. 4	" "	12.10 P.M.	64	37.12	20.3	404	390	28.3	29.0	7.0	7.1
29	" "	3.40 "	61	37.50	22.3	422	423	22.7	23.6	5.4	5.6
May 13	" "	11.30 A.M.	58	37.64	21.5	417	412	28.1	28.5	6.7	6.9
Nov. 19	" "	3.30 P.M.	72	37.50	20.0	420	407	24.8	22	5.9	5.4
										6.0	**6.1**
May 30	Pike's Peak	11.40 A.M.	70	37.56	17.7	409	414	23.4	26.8	5.7	6.5
31	" "	10.25 "	68	37.33	20.4	419	404	48.9	45.5	11.7	11.3
June 1	" "	8.10 P.M.	78	37.72	19.6	427	420	52.8	57.7	12.3	13.7
2	" "	10.25 A.M.	68	37.72	20.8	410	409	31.6	35.0	7.7	8.6
Oct. 24	" "	9.30 "	88	37.58	21.0	431	430	42.5	44.1	9.9	10.3
										9.5	**10.1**

Eager											
Oct. 3	Colo. Springs	3.00 P.M.	76	36.50	19.6	450	440	18.0	17.5	4.0	3.9
Nov. 20	" "	10.00 A.M.	80	37.00	21.5	395	427	29.7	29.9	7.5	7.0
26	" "	3.30 P.M.	89	37.44	19.5	405	388	23.6	19.1	5.8	4.9
										5.8	**5.3**
Oct. 25	Pike's Peak	10.30 A.M.	106	36.94	20.2	395	396	35.4	33.2	9.0	8.4
Munro											
Oct. 3	Colo. Springs	5.00 P.M.	68	37.84	19.9	444	409	22.7	18.9	5.1	4.6
10	" "	3.00 "	70	37.22	22.0	444	430	32.2	29.0	7.3	6.7
Nov. 14	" "	7.00 "	74	37.44	20.9	460	449	35.1	34.1	7.6	7.1
										6.7	**6.1**
Oct. 24	Pike's Peak	12.10 P.M.	96	37.39	20.6	446	436	33.0	29.7	7.4	6.8
25	" "	9.00 A.M.	96	36.84	18.6	462	427	46.0	40.0	10.0	9.4
										8.7	**8.1**
Robison											
May 1	Colo. Springs	9.05 A.M.	70	37.12	21.9	448	437	38.9	37.0	8.7	8.5
May 31	Pike's Peak	9.25 A.M.	90	37.61	18.3	417	400	49.8	49.4	11.9	12.4
June 1	" "	7.40 "	85	37.25	20.6	418	395	55.1	50.4	13.2	12.8
										12.6	**12.6**

for the right hand and 53 per cent for the left hand. The higher rate in him was undoubtedly largely due to the increase in heart rate, the pulse pressure averaged 34 mm. at both altitudes. The determination on May 30 occurred during an attack of mountain sickness while arterial pressures were extraordinarily high, systolic 138 mm. and pulse pressure 44 mm. At that time the venous pressure also reached a maximum of 20.6 cm. of water.

A greater fluctuation occurred in the blood flow of Sisco while on Pike's Peak than in any other of our subjects. His pulse rate was also more changeable on the summit than in Colorado Springs. The first determination made on him twenty-four hours after the ascent in May showed no increase whatever. At that time his pulse rate was greater than it generally was in the blood flow studies in Colorado Springs. However, his systolic pressure was only 110 mm., the lowest found in him, and the pulse pressure was also unusually low, only 26 mm.; his average being 32 mm. The blood flow per 100 c.c. of hand volume was almost 100 per cent greater the following day, May 31, with the pulse rate practically the same. There was, however, a slight rise in arterial pressure, the systolic pressure was 114 mm. and the pulse pressure 31 mm. The maximum flow, on June 1, occurred at a time when the systolic pressure was 120 mm., the highest obtained on him on the Peak; but the pulse pressure, 34 mm., was not equally raised. With these changes was associated a moderate acceleration of the heart rate. Sisco showed at the high altitude an average increase of 58 per cent for the right and 66 per cent for the left hand.

Our study of Eager and Munro was unfortunately brought to a sudden end on the Peak by the breaking of the glass flasks used for measuring the water for the calorimeters. Two determinations were made on Munro and only one on Eager. The first on Munro, like the first for Sisco, gave no change; the second, however, gave an increase over the Colorado Springs rate of 54 per cent for the right and 49 per cent for the left hand. His average percentage increase for the two determinations was right 30 and left 33. The determination of blood flow for Eager made two days after the ascent showed an acceleration of 55 per cent for the right and 58 per cent for the left hand.

The observations on Robison, while too few to be wholly satisfactory, are of more than passing interest because he had resided for more than four weeks on the summit at the time his circulation rate was determined. It is interesting, therefore, to find that he too had an augmented flow; 45 for the right and 48 per cent for the left hand. This is somewhat less than that found for other subjects. His pulse rate was 20 and 15 beats more rapid than during the experiment in Colorado Springs. The differences in the arterial pressures are of interest, on May 1 while in Colorado Springs the systolic was 114 mm. and the pulse pressure 30 mm.; during both observations of the blood flow on the summit the systolic was 110 mm. and the pulse pressure only 26 mm. If the pulse rate be multiplied by the pulse pressure and the product be taken as a measure of the volume per minute of the blood stream, it will be found that the increase on May 31 should have been only 18 per cent and on June 1 12 per cent. This fact suggests the occurrence of vasomotor changes that our methods have failed to detect.

The augmentation in blood flow found to occur in all of our subjects on Pike's Peak is greater than in other oxygen-want adaptive changes. It should here be noted that in calculating the data for the blood flow we have not corrected the specific heat of the blood constant for the increase in red corpuscles on Pike's Peak. This correction has been neglected because the number of corpuscles was constantly increasing and further it would only add slightly to the figures recorded for the flow. That the average flow would decrease somewhat with longer residence on the Peak is suggested by the observations on Robison and by the experience of the English-American Pike's Peak Expedition[1] in which they found the pulse rate to be slightly reduced from the maximum after a residence of two weeks.

On considering all of our data we are forced to conclude that the facts of the blood pressures and changes in pulse rates do not wholly account for the augmentation in the blood flow observed at the high altitude. There were vasomotor changes for which we cannot account. It was our experience during both expedi-

[1] Douglas, Haldane, Henderson, and Schneider: Philosophical Transactions of the Royal Society of London, 1913, Series B, ciii, p. 270.

tions that each of us was more sensitive to heat on the mountain than we were at 6000 feet and that we felt comfortable at a lower room temperature. On looking over the room temperatures experienced during the experiments on Havens, Schneider, and Sisco we fail to find here an explanation of the variations. For none of these three men was the most rapid flow coincident with the highest room temperature. The slowest rate for Sisco was associated with the minimum room temperature; but this was not the case for the others. It appears, therefore, that room temperature does not explain the vasomotor changes.

That the body temperature was not instrumental in altering the vasomotor relations is also evident when the more extended records of Havens, Schneider, and Sisco are examined. Havens had one of the lower rates of blood flow on the day his body temperature was the highest, 38° C. For Schneider the more rapid rates occurred when body temperature was the highest, but the small increase in temperature of 0.23 of a degree would very likely be ineffective as a stimulating factor. With Sisco the maximum rate occurred on a day when his body temperature was also maximum, but the next day while the body temperature was still the same the rate of blood flow had lowered 37 per cent.

It is now generally recognized that the rate of flow of the blood from arteries to veins does not depend upon the arterial pressure alone but that two other factors, the caliber of the vessels between artery and vein, and the venous pressure, are of prime importance. We have shown in our former paper[1] that the venous pressure of all our subjects, with the exception of Schneider, was decidedly lowered — 25 to 87 per cent — on Pike's Peak. The fall in venous pressure along with the acceleration of pulse rate will in large part account for the observed increase in the rate of blood flow at the high altitude. However, there are some irregularities involving an increase in the caliber of the arterioles for which we do not account.

It would be interesting for such a study as ours to have a subject who reacted as did Haldane,[2] whose pulse rate was from 15 to 20 beats slower on Pike's Peak than at sea-level. From a

[1] Schneider and Sisco: *loc. cit.*

[2] Douglas, Haldane, Henderson, and Schneider: *loc. cit.*, p. 265.

study of his pulse rate and amplitude of heart beat it was concluded that his circulation rate was decidedly decreased at the high altitude. Very likely his venous pressure lowered with residence on Pike's Peak but it is unlikely that the circulation rate was unaltered and certainly it appears that it could not have been accelerated.

The Influence of Oxygen Inhalation on the Heart Rate

If it is the oxygen-want at low barometric pressures that is responsible for the accelerated heart action and the observed increase in the rate of the flow of blood through the tissues, it is probable that inhalation of oxygen may so benefit the body that the heart rate will be retarded and the blood flow diminished. We have tested the influence of oxygen inhalation on both the pulse rate and blood flow.

One of us[1] reported from a series of studies on Mr. Robison, the resident manager of the Summit House on Pike's Peak, that the breathing of oxygen-rich mixtures caused in him, when on the summit, a marked slowing of the pulse rate. It was suggested that the effect of the oxygen was exerted through the oxidation of certain easily oxidizable metabolites normally present in a considerable amount in the blood at very high altitudes. It was also pointed out that these readily oxidizable metabolites accumulate in the blood because of the deficient supply of oxygen and that they may in turn accelerate the heart rate.

We have now compared the influence of oxygen on the pulse rate in Colorado Springs and on Pike's Peak on six subjects and have found the slowing action in each to be more pronounced on the Peak. For this study the subject sat quietly for ten or more minutes, or until the heart rate became constant. The oxygen was then administered by means of a small closed apparatus provided with a soda-lime chamber, for the absorption of carbon dioxide, and with a rubber balloon for the reception of the tidal air and the reserve supply of oxygen. The oxygen was introduced continuously at a uniform rate which was determined by the need of the subject. The oxygen was pure, made from ozone. All

[1] Schneider: This journal, 1913, xxxii, p. 300.

air was thoroughly washed out of the apparatus with the oxygen before each experiment. The mouthpiece and all tubes were of a large size so that the resistance was reduced to a minimum and the breathing was comfortable. The oxygen was generally administered for ten minutes. The pulse was counted every minute during oxygen inhalation and for an equal interval on return to air. The results of the experiments are given in Table II.

It will be seen that the amount of slowing in Colorado Springs varied from 2.5 to 8.8 per cent. The extent of slowing was not determined by the heart rate at the time the oxygen was administered; thus in the case of Havens in one experiment with a cardiac rate of 80 a reduction to 78 or only 2.5 per cent occurred, while at another time with an initial rate of 62 the heart responded with a slowing of 4.8 per cent. Individual differences appear among the records in Colorado Springs, thus the retarding effect of the oxygen was less pronounced for Havens than for Sisco, Robison, or Munro. The average reduction obtained for the six subjects in Colorado Springs was 5.4 per cent.

Many oxygen breathing tests have been made with men in Colorado Springs and all, with one exception, have responded with a definite, though usually slight, slowing of the heart. The exception was Robison immediately after his descent from the Peak in November, 1912; the case has been discussed by Schneider.[1] At that time the partial pressure of oxygen in Robison's blood was extraordinarily high because he continued for a time to ventilate his lungs as thoroughly as on the Peak and this resulted in an alveolar oxygen pressure at least 10 mm. above that found in men acclimatized to the altitude of Colorado Springs.

On Pike's Peak we were able to reduce the pulse rate in our six subjects from 7.4 to 20.8 per cent. The least reduction, that of 7.4 per cent. occurred in Havens at a time when his heart rate had not taken up the high tempo of the later days. It will be recalled that his pulse rate did not clearly accelerate on the Peak for forty-eight hours. All other tests with oxygen on the mountain show a retardation of 10 or more per cent. The average amount of slowing for all our subjects while on the Peak was 14 per cent.

Since the cardiac rate may be so decidedly slowed at an

[1] SCHNEIDER: *loc. cit.*

TABLE II

SLOWING THE HEART WITH OXYGEN

Subject	Date	Place	Pulse rate with air	Pulse rate with O_2	Percent. of slowing
Havens	Apr. 17, 1913	Colo. Springs	62	59	4.8
"	May 8	" "	68	64	5.9
"	21	" "	70	67	4.3
"	Nov. 19	" "	80	78	2.5
"	May 30	Pike's Peak	68	63	7.4
"	31	" "	79	70	13.0
"	Oct. 25	" "	92	82	10.9
Schneider	May 21	Colo. Springs	76	74	2.6
"	Nov. 14	" "	80	75	6.3
"	Dec. 18	" "	82	78	4.9
"	May 30	Pike's Peak	90	79	12.2
"	31	" "	92	80	13.0
"	Oct. 24	" "	108	96	11.1
"	25	" "	102	88	13.7
Sisco	May 8	Colo. Springs	64	59	7.8
"	21	" "	62	59	4.8
"	Nov. 19	" "	72	67	6.9
"	May 30	Pike's Peak	72	62	13.9
"	31	" "	70	63	10.0
"	Oct. 24	" "	88	78	11.4
Robinson	Oct. 13, 1912	" "	80	64	20.0
"	14	" "	82	70	14.6
"	May 30, 1913	" "	88	70	20.4
"	1	Colo. Springs	68	62	8.8
Munro	Oct. 24	Pike's Peak	96	76	20.8
"	Nov. 14	Colo. Springs	72	66	8.3
Eager	Oct. 24	Pike's Peak	106	88	17.0
"	Nov. 14	Colo. Springs	88	84	4.5
"	Dec. 18	" "	90	87	3.3

altitude of 14,109 feet by the breathing of oxygen-rich mixtures, the conclusion follows that the quickened action of the heart in rarefied air is a means of compensating for the lack of oxygen.

Arterial pressures were determined in some of the oxygen inhalation experiments, both in Colorado Springs and on Pike's Peak, but no definite evidence of change was obtained. Unfortunately no determinations of venous pressure under these conditions have as yet been made.

The Influence of Oxygen Inhalation on the Blood Flow

We have made a partial study of the influence of the inhalation of oxygen on the blood flow in the hands. Unfortunately this work was interrupted when we were last on Pike's Peak by an accident to our apparatus. Consequently certain control tests we had planned have had to be omitted. However, since the data obtained are on the whole concordant we venture to make a tentative report at this time.

The normal rate of the blood flow was determined for ten or more minutes, this was followed by another period of at least ten minutes during which the subject breathed oxygen through the apparatus used in slowing the heart rate, and then occurred an after air period of the same length of time. In six out of seven experiments on the Peak there was a diminution in the rate of the blood flow during the oxygen inhalation period. Two protocols giving calorimeter readings and pulse rates are cited to show the sequence of the changes.

Eager. Mouth temperature, 36.56°. Rectal temperature, 36.94°. Volume of the hand 396 c.c. Hand put into the calorimeter containing 3015 c.c. of water at 10.46 a.m. Following Stewart's suggestion for computation we find that during the first air breathing period of ten minutes there was a flow of 33.2 gm. per minute for the entire hand and 9 gm. per 100 c.c. of hand per minute. In the oxygen breathing period there were 27.5 gm. for the hand and 7 gm. for 100 c.c. of hand volume. During the after period these were 36.1 and 9.1 gm. each.

Sisco. Mouth temperature, 36.61°. Rectal temperature, 37°. Volume of the hand 430 c.c. Hand put into the calorimeter containing 3015 c.c. of water at 9.34 a.m. During the first period the flow

for the entire hand was 44.1 gm. of blood per minute or 10.3 gm. per 100 c.c. of hand per minute. For the oxygen inhalation period the flow for the whole hand was 39.9 gm. and 9.3 gm. for 100 c.c. of hand per minute. In the after period these figures are 45.9 gm. and 10.7 gm. respectively.

Eager				Sisco			
Time	Calori-meter	Pulse Rate	Notes	Time	Calori-meter	Pulse Rate	Notes
10.50	31.49	106	Air breathing	9.38	29.78	88	Air breathing
10.52	31.52		Room 20.1	9.40	29.91		Room 21.4
10.54	31.63	105		9.42	30.04		
10.56	31.69			9.44	30.17		
10.58	31.73	106		9.46	30.26	88	Room 21.4
11.00	31.79		Oxygen on	9.48	30.41		Oxygen on
11.02	31.81	92		9.50	30.48	80	
11.04	31.89	88	Room 20.1	9.52	30.54	76	
11.06	31.93	88		9.54	30.66	78	
11.08	31.98	84		9.56	30.81	78	Room 21.6
11.10	32.00	88	Oxygen off	9.58	30.91	78	Oxygen off
11.12	32.05	90		10.00	31.03	84	
11.14	32.11			10.02	31.12	88	Room 20.9
11.16	32.18	106		10.04	31.28		
11.18	32.22		Room 20.2	10.06	31.39		
11.20	32.29			10.08	31.44		Hand out
11.22	32.33		Hand out	10.18	31.33		Room 20.5
11.32	32.21						

It should be noted that the diminution in the flow of the blood in the hands during oxygen inhalation was immediate and was in general, as for Sisco, most marked the earlier minutes of the period. The slowing of the heart ordinarily took place more

gradually and did not tend to return to the normal rate as did the flow of the blood in several instances.

TABLE III

BLOOD FLOW IN 100 C.C. OF HAND DURING OXYGEN INHALATION ON PIKE'S PEAK

Subject	Date	Blood flow in grams per Minute			Slowed with O_2 %	Heart slowed with O_2 %
		Fore-period	Oxygen-period	After-period		
Eager	Oct. 24	8.4	7.0	9.1	16.7	17.0
Havens	24	11.8	9.5	13.5	19.5	10.9
Munro	24	7.4	7.0	7.0	5.7	20.8
"	25	10.0	9.1	10.0	9.0	—
Schneider	24	10.3	9.9	10.5	3.9	11.1
"	25	11.0	10.9	11.9	0.9	13.7
Sisco	24	10.3	9.3	10.7	9.7	11.4

A summary of all the observations on the influence of oxygen on the blood flow made on Pike's Peak is given in Table III. The retardation in the flow in the six positive experiments ranged from 3.9 to 19.5 per cent. The diminution in the rate of flow was not parallel with that of the pulse rate. Generally the percentage decrease in the blood flow was less than that of the pulse rate. In Havens, however, the flow of blood was reduced 19.5 per cent while his heart rate decreased only 10.9 per cent. A satisfactory explanation of these differences is not at hand. In our records of the heart slowing we find that for each subject, except Havens, the pulse became fainter and usually felt softer during oxygen inhalation, while on the contrary Havens' pulse became harder and more pronounced. The change in the character of the pulse, in all cases except Havens, suggests a decrease in the heart amplitude as an explanation of the diminished blood flow. Attention is called to the fact that in five out of the seven experiments there was a greater flow in the after-period than in

the fore-period. A detailed study of these various irregularities will be made in a future expedition to Pike's Peak.

In Colorado Springs experiments made on five of our subjects have failed to show a slowing of the blood stream when the subject was in a normal condition. Thus in one series of oxygen inhalation experiments we obtained the following rates per minute: — Eager, fore-period 7.5 gm., oxygen-period 8.4 gm.; Havens, fore 4.9 gm., oxygen 4.8 gm.; Munro, fore 7.7 gm., oxygen 7.8 gm.; Schneider, fore 4.5 gm., oxygen 5.1 gm.; and Sisco, fore 5.9 gm., and oxygen 6.5 gm. We have, however, two experiments made in Colorado Springs in which a diminution in the flow occurred during oxygen inhalation. One of these was with Eager one hour after he had pushed a heavy motorcycle several miles on a dusty road, then the rate of blood flow per minute in 100 c.c. of hand was in the fore-period 10.1 gm., during the oxygen inhalation-period 8.1 gm., and in the after-period 9.4 gm. The second experiment was with Schneider at the end of a very busy day. Then the blood flow in his hands averaged 8.4 gm. in the fore-period, 7.6 gm. in the oxygen-period, and 8.1 gm. during the after-period.

The influence of oxygen on the rate of the blood flow at the high altitudes is the opposite of that shown by Stewart[1] in a case of cyanosis in which inhalation of oxygen increased the flow of the blood by an amount varying from 30 to 70 per cent. It should here be noted that Stewart found the breathing of oxygen did not change the rate of the blood flow in the hands of two normal men at a low altitude. Our results in part resemble those obtained by Stewart[2] on two healthy men during forced breathing. A distinct diminution in the flow through the hands was observed by him during the periods of increased respiration. We have tried to avoid forced breathing in our experiments. Since the conditions of experimentation were the same in Colorado Springs and on Pike's Peak, and at the lower altitude ordinarily no change occurred, we believe that forced breathing may be eliminated as an explanation of our results. A difference in the reaction will

[1] Stewart: Journal of pharmacology and experimental therapeutics, 1911, ii, p. 477.

[2] Stewart: This journal, 1911, xxviii, p. 190.

be observed on comparing Stewart's and our data. In forced breathing the change in blood flow required some minutes to reach its maximum while with our oxygen experiments it was almost immediately maximal. We also found that the heart rate increased during the forced breathing while in our experiments with oxygen inhalation the rate decreased.

The above experiments dealing with the effects of oxygen inhalation on the heart rate and the velocity of blood flow indicate that it is the lack of oxygen at high altitudes that calls forth the changes within the circulatory system. Alterations in the composition of the blood very likely influence the heart and the vasomotor mechanism. Durig and Kolmer[1] after finding the heart rate to be permanently augmented by residence on Monte Rosa question as to the causes of acceleration. It will be recalled that they found the heart rate accelerated for two or three days following ascent; after which there was some retardation, but never a return to the rate generally found at low altitudes. They associated the early augmentation in part with an increased body temperature, and in part with the altered composition of the blood resulting from the lack of oxygen. They assumed that the retardation was caused by an increased tone of the cardio-inhibitory center and that this heightened tone was associated with the more intense stimulation of the lung fibers of the vagus nerve in consequence of increased lung ventilation. We as yet are not prepared to debate the questions as to the cause or causes of the acceleration or how the stimulus acts. A study of these questions is in progress and the investigation will be continued in another and longer expedition to Pike's Peak. It here should be noted, however, that our temperature records (see table on the blood flow) fail to support Durig and Kolmer's contention that a rise in the body temperature causes, in part or wholly, the initial augmentation in heart rate. Our men did not have a higher temperature on Pike's Peak than in Colorado Springs.

Attention is called to the fact that with oxygen we slowed the heart of Robison, whom we take it was fully acclimatized to the

[1] Durig: Physiologische Ergebnisse der im Jahre 1906 durchgeführten Monte Rosa-Expedition, p. 48.

altitude of 14,109 feet, as much as in any, and more than in the majority, of the members of our expeditions. Unfortunately we did not test the influence of oxygen on the rate of blood flow with Robison.

Summary

1. The rate of blood flow in the hands of the six men examined was increased by residence on Pike's Peak by an amount varying from 30 to 76 per cent. The increase in the rate of flow has been associated in part with an augmented rate of heart beat and a fall in the venous pressure, also in part with a dilatation of the arterioles.

2. The breathing of an oxygen-rich mixture slowed the heart rate in each of the six subjects to a greater degree on Pike's Peak than in Colorado Springs. The average retardation was 14 per cent at 14,109 feet, and 5.4 per cent at 6000 feet.

3. The arterial pressure was not clearly altered at either altitude during oxygen inhalation; but the pulse, with one exception, was fainter and softer.

4. Oxygen inhalation diminished the rate of blood flow in the hands from 4 to 20 per cent on Pike's Peak while the flow was not ordinarily altered in Colorado Springs.

5. In view of the beneficial influence of oxygen inhalation — the retardation of the heart rate and diminution in the rate of blood flow — it was concluded that oxygen-want induces the adaptive high altitude circulatory changes.

A STUDY OF THE MECHANISMS BY WHICH MUSCULAR EXERCISE PRODUCES ACCELERATION OF THE HEART

By H. S. GASSER and WALTER J. MEEK

[*From the Physiological Laboratory of the University of Wisconsin*]

THE mechanisms which have been described as concerned in acceleration of the heart during exercise may be divided into two classes: first, those depending on the physical and chemical changes in the blood, and, second, those depending on nervous impulses of either central or peripheral origin. Most work on the subject has favored the latter class of mechanisms. Petersen and Gasser[1] have recently found that the rate of excised hearts is not increased by extracts of fatigued muscles although the amplitude may become greater, while Athanasiu and Carvallo[2] long ago showed that acceleration might be purely nervous in origin. This was done by binding the arm with an Esmarch bandage. On working a dynamometer with the hand thus rendered anaemic the heart accelerated from 10 to 20 beats per minute. These authors felt that if the chemical products of muscular contraction affected the heart it was only after prolonged exercise and the action was more nearly pathological than physiological.

That the acceleration in exercise is not due to chemical or physical changes in the blood but rather to nervous phenomena of some kind is also indicated by the interesting observations of Bowen[3] and Buchanan.[4] Bowen studied the latent period of the acceleration following the onset of exercise. He determined by continuous graphic records of the pulse that the diastole of the next pulse cycle occurring after the commencement of exercise is materially shortened, and he therefore gave the latent period as one cardiac cycle. Buchanan, by measuring the length of the heart cycle with the capillary electrometer, also found that the acceleration occurs so promptly that even if the muscular contraction takes place at the end of a systole the immediately en-

suing diastole of the same cycle is shortened. In one case the cycle was shortened to 81 per cent of the preceding. We have confirmed these results by electro-cardiographic methods. The duration of the pulse cycle was obtained with great accuracy on a rapidly moving photographic registration apparatus, and the exercise, which consisted in clenching the fist, was simultaneously recorded by a tambour connected with an aspirator bulb held in the hand clenched. One of our records shows that the first cycle following the clenching of the fist was shortened 9 per cent and the second 25 per cent. It took exactly 1.09 seconds for this 25 per cent increase in rate to be brought about.

Inasmuch as the latent period for the acceleration of the heart has been proved to be one cardiac cycle or less, the possibility is at once eliminated that chemical products of muscular metabolism or the heat evolved in such metabolism could have had time to reach the heart. The first acceleration in exercise can therefore scarcely be due to any direct action of metabolites on the heart or cardiac centres, or to a reflex arising from stimulation of sensory nerves in the heart by heat, as is maintained by Mansfield,[5] or to inhibition of the vagal centre by afferent impulses from the lungs arising from increased respiration, as is maintained by Hering.[6] Apparently the only mechanism in the body that could act within the specified time limits is one that is purely nervous in character.

The results just detailed give considerable support to Johannson's[7] theory that impulses along the motor paths affect the cardiac centres of the medulla. This author found that tetanization of the hind limbs by stimulation of the severed spinal cord produced a cardiac acceleration that was very small compared with that occurring in normal voluntary movement. He attributed this difference to a psychic stimulation of the accelerator centre occurring during the voluntary activity, strengthening his conclusions by the observation that passive movements of the limbs in the normal animal produce very little acceleration. Johannson gave no reason for his assumption that the nervous action was on the accelerator centre rather than on the cardio-inhibitory.

Athanasiu and Carvallo[2] advanced an entirely different expla-

nation for acceleration in exercise which was based on the following observations: First, that when paraplegic individuals were ordered to make voluntary efforts to move their disabled limbs no acceleration resulted. Second, that on mechanically exciting an animal poisoned with chloralose the resulting muscular activity produced an increased heart rate, but if no movements resulted the acceleration was absent. They concluded from these by no means conclusive experiments that the voluntary motor impulse is alone insufficient to cause an acceleration of the heart and that reflex movements very efficiently produce it. In this way they came to the view that the working muscles send excitations toward the higher centres, which in their passage through the medulla depress the cardio-inhibitory centre. Athanasiu and Carvallo based their conclusion that it is the cardio-inhibitory centre that is concerned upon some experiments on animals a few hours after vagotomy. Pulling rhythmically on the hind limbs caused the animals to withdraw them actively, but the movements now produced no acceleration, although the same manipulation did increase the heart rate of the normal animal.

Hering[6] studied the role of the extrinsic cardiac nerves in motor accelerations of the heart by determining the heart rate as the result of exercise before and after removal of the stellate ganglia. He found that after removal of these ganglia the rate following exercise was considerably less than normal, while the resting rate was considerably higher. The acceleration was, therefore, much reduced. As a result he arrived at the conclusion that the increase in heart rate in muscular activity is principally dependent on the integrity of the accelerator nerves. This he believed to be supplemented especially in the early stages of exercise by diminution of vagal tone, but only in so far as this is brought about by the increased respiratory rate resulting from the increased muscular activity.

The mechanism of reflex cardiac acceleration due to stimulation of sensory nerves has been investigated by several workers and the results have been rather freely applied in explaining increased heart rate during exercise. MacWilliam[8] came to the conclusion that reflex acceleration is dependent on depression of the vagus centre, since it occurs with remarkable suddenness, rises rapidly

to its maximum, remains essentially the same after section of the accelerators, and since direct stimulation of the accelerators is followed by an increase in rhythm only after a long latent period. A number of years later this same field was covered independently by Hunt,[9] whose work confirmed that of MacWilliam's. Hunt showed that in reflex acceleration the response is the same as that occurring on cutting the vagi; that is, the diastole is markedly shortened. This is in marked contrast to the results obtained by accelerator stimulation, in which case both systole and diastole were shortened.

Aulo[10] suggested that by Hunt's criterion for depression of the cardio-inhibitory centre the nature of acceleration in exercise might be determined from the sphygmogram. Curves were therefore taken and it was shown that in exercise for periods of one minute, at least, the diastole was the part shortened. Aulo from these observations concluded that if Hunt's criterion is correct the first increase in pulse rate results from a diminution of vagal tone.

In the light of our present knowledge it seems that acceleration of the heart in exercise is best attributed to nervous mechanisms. Although experimentally it is clear that reflex acceleration may be produced either by depression of vagal tone or stimulation of the accelerators,[20] workers are not yet agreed as to which of the two centres is normally more affected, no matter whether the stimulus should prove to be an irradiation from voluntary impulses, or a sensory one from peripheral parts.

Our own work was taken up primarily to determine the relative parts played by the vagi and accelerators in cardiac accelerations during the early stages of exercise. The investigation has been carried out according to the following scheme. Accelerations occurring in exercise were determined: (1) When both the accelerator and inhibitory mechanisms were intact; (2) when the accelerator mechanism was eliminated and the inhibitory mechanism was intact; (3) when the inhibitory mechanism was eliminated and the accelerator intact; and (4) when the heart was freed from all extrinsic nervous control. An inquiry was also made in regard to factors causing accelerations after all the extrinsic nerves had been eliminated.

Methods

The experiments were performed entirely on dogs. It was first necessary to get the pulse of the normal dog at rest. To do this the dog was made to lie quiet until the pulse rate no longer decreased. The rectal temperature was then taken. The dog was exercised for two minutes, the exercise consisting in making the dog run as fast as possible at the end of a leash. The pulse was counted immediately at the end of exercise, the loss of time being only a few seconds, and the counting was continued in most cases until the rate returned to normal. The temperature of the animal was again observed. After the temperature returned to normal, atropine was administered subcutaneously to determine the power of the heart to accelerate after complete removal of vagal inhibition. To insure a complete block of the vagal endings 1.5–2 mg. of atropine were administered subcutaneously and after the pulse had reached its maximum an additional half-milligram was given to see if the pulse could be driven any higher. In most cases it was found that after administration of atropine the pulse rate rose rapidly to a maximum, then slightly decreased, remained at this level for a short time and then again gradually decreased. No further injection of atropine could then increase the pulse rate. This decrease in rate must be due to the inability of the heart to maintain such a high frequency, since it has been our experience that several hours after administration of atropine electrical stimulation of the vagus was without effect on the heart. The high pulse rate was readily counted by means of a phonendoscope placed over the chest at the position of the apex beat.

The next step was the removal of the accelerator nerves. The technique may be of use to other workers and will be described in some detail. It will be well to briefly review the anatomy of the ganglion in the dog, especially since it was found to be given incorrectly in some recent textbooks of physiology. As usually seen from the open thoracic cavity it is a white, laterally compressed bean-shaped body shining through the pleura at about the level of the second rib. It lies at the edge of the longus colli muscle with the smallest diameter in the sagittal plane. From

the upper pole, which lies anteriorly and ventrally, the two branches of the ansa subclavia are given off. These pass ventrally one on each side of the subclavian artery to the inferior cervical ganglion. From the anterior portion of the ganglion branches pass to their distribution in the nerves of the brachial plexus. From the dorsal edge arise the rami communicantes to the anterior divisions of the first and second dorsal nerves. The ramus to the third dorsal segment may arise directly from the stellate ganglion, but more often it first joins the sympathetic chain. Posteriorly the ganglion tapers out and is connected with the sympathetic chain at the level of the third rib.

The operations were performed under ether anaesthesia with due aseptic precautions. The incision was made in the midline from the level of the larynx to the suprasternal notch, and the skin separated from the sterno-hyoid and the sterno-thyroid muscles. A separation was made between these two muscles and the carotid sheath exposed. The vagus nerve was then freed from the sheath and its course followed to the inferior cervical ganglion, from which arise the ansa subclavia. The field of operation was most easily exposed by passing the forefinger behind the carotid sheath down into the superior mediastinal space to the level of the third rib. The pleura was carefully lifted from its position over the ganglion. The field was then further exposed by inserting two large flat-bladed retractors into the opening prepared by the finger. With these the subclavian vessels were displaced ventrally and a retractor placed medially served to hold the carotid and vertebral arteries out of the field.

From this time on illumination from a head mirror was necessary. When the ansa subclavia had been found a Halsted mosquito clamp was placed on the dorsal loop and by gentle traction its course was followed to the antero-ventral pole of the ganglion. At this point the rami communicantes to the brachial plexus were encountered. One of the larger of these was secured by a mosquito clamp which was subsequently used as a guide. Both branches of the subclavian loop were then cut, after which each of the branches to the brachial plexus was in turn exposed and severed with a cutting probe. The position of the rami communicantes to the first and second dorsal nerves was de-

termined and the rami cut. The removal was completed by severing the connection of the ganglion with the sympathetic chain. Care was taken throughout the operation not to produce a pneumothorax by puncturing the pleura, and on the left side the thoracic duct had to be avoided. When its position had been determined it was easily displaced forward with a retractor. The wound was closed by uniting the sterno-hyoid and the sterno-thyroid muscles and then expressing the air from the mediastinum through the suture by pressure on the outside of the thorax. The skin incision was closed by a continuous suture and the neck bandaged without further dressing.

The animals made uneventful recoveries and they were playful and ate well. The only external sign of the removal was the fact that the nictitating membrane extended out over the bulbus oculi, due to its loss of tone after cutting the sympathetic innervation of its retractor muscle. This condition persisted although the membrane later developed a certain amount of tone. The dilation of its vessels which occurred at first usually entirely disappeared.

The completeness of the removal was determined by autopsy in every case. Usually the removal was found to be complete. The ganglion was in most cases found to have been separated from the sympathetic just above the first dorsal ramus. In some cases a tiny speck of the ganglion was found adherent to the third ramus. In some of the earlier operations before the technique was perfected, autopsy showed that the knife had slipped into the ganglion and that portions remained adherent to the second or second and first rami. In no case was there a trace of the antero-ventral half of the ganglion or the cardio-accelerator nerves which arise from it. Data from the earliest experiments in which the autopsy showed incomplete removal of the ganglion were, of course, discarded. In addition to the operations described above several control experiments were made which will be referred to in the text.

After recovery the resting pulse rate of the dog was counted as before and the reaction of the heart to the two-minute period of exercise was determined. The resting pulse was also determined when the vagal endings were blocked by atropine. These obser-

vations were made over periods ranging from sixteen hours to five months after the operation. The vagi were then divided in the neck and similar observations repeated.

I. Effects of Removing the Stellate Ganglia

In six out of nine attempts the autopsies showed that we had succeeded in completely removing the stellate ganglia. Data from the three failures were, of course, rejected. Before the subsequent vagotomies were performed the animals were observed during periods varying from one to nineteen weeks. The principal data will be discussed under the following sections:

1. The resting pulse. — In every case the resting pulse after removal of the accelerators was less than in the normal animal. The pulse rate fell rapidly immediately after the operation and then more gradually decreased until it reached a level at which it remained nearly constant. The immediate decrease in rate was usually over one-half the total. By the end of about the first week following ganglionectomy the rate had slowly fallen to a level which was fairly constantly maintained. Apparently the large decrease at first was due to the removal of the tonic accelerator action and the subsequent more gradual decrease was due to the return of the normal tone to the vagi. The decrease from the normal rate differed widely in the different animals, depending mainly on the rate of the normal resting pulse. In dogs 3 and 5, in which the normal resting pulse was high, 140 and 136 per minutes respectively, the corresponding resting pulses after the ganglionectomy were 76 and 80, while in dog No. 4 the pulse which was normally 78 fell only to 66. The actual decrease thus varied all the way from 12 to 64 per minute.

So far as the nervous mechanisms are concerned the resting pulse rate might be determined in three different ways, assuming that the inherent automaticity of the heart is fairly constant. Some evidence for such an assumption has been presented by Stewart and Pike,[11] who found that the heart rate of cats during cerebral anaemia was remarkably uniform. In the first place the accelerator tone might be a constant factor for the species and the variations in rate be due to vagal activity; in the second

TABLE I

Showing acceleration before and after removal of the stellate ganglia. The resting pulse rates are in each case the lowest found in a series of observations. The accelerations given were observed in the same experiment as that in which the corresponding resting pulse rate was obtained. In experiment No. 5 the rate after removal of the ganglia finally fell to 80 but acceleration was not measured at this time. Rates are given in beats per minute.

	Before removal			After removal		
Ex.	Pulse at rest	Acceleration	Per cent	Pulse at rest	Acceleration	Per cent
1.	116	44	37	70	42	60
2.	98	50	51	66	48	72
3.	140	44	31	76	50	65
4.	78	52	66	66	36	34
5.	136	38	28	96 80[1]	34 —	— —
6.	88	40	45	72	40	35

[1] The pulse finally fell to 80 in this dog.

place the vagal tone might be constant and variations be brought about by differences in accelerator action; in the third place each of these factors might be variables. Our data seem to enable us to decide between these possibilities.

The normal resting pulse rates of the dogs were respectively 116, 98, 140, 78, 136, and 88 per minute. After the operation the corresponding pulse rates finally fell to 70, 66, 76, 66, 80, and 72. These figures show that after the accelerator action was removed the heart rate became remarkably uniform; the normal pulses differed as much as 62 per minute, while after removal of the accelerators there was a difference of only 14 per minute. If the accelerator action had been a constant and the vagal action variable, then the resting rate after the operation would have varied according to the previous normal resting rates. As this was not the case it is evident that in extirpating the accelerator nerves the variable factor was removed, and the conclusion seems therefore justified that the variations in the resting pulse rates of

our six dogs were in a larger measure dependent on the activity of the accelerators than on any vagal action.

2. The reaction to muscular exercise. — The ability of the heart to accelerate during muscular exercise after removal of the stellate ganglia was striking. The actual increases in rate per minute before and after the operation were not markedly different. Table I gives the exact figures. If the lowest observed resting rate in each experiment and the corresponding acceleration are compared it will be noted that in one case the acceleration was greater after the operation, in one case the same, and in four cases slightly less. On looking over all observations made it has been found that in the majority of cases the accelerations were a few beats less per minute after removal of the accelerators.

The results obtained show conclusively that the accelerator mechanism is by no means necessary to secure the increase in pulse rate normally occurring at the beginning of ordinary exercise. This conclusion is directly opposite to that reached by Hering.[6] If we compare our results with his the reason for this becomes at once apparent. In our experiments the resting pulse after the operation was in every case much lower than in the normal animal, and this lower rate was maintained until the death of the animal, one dog being observed for a period of five months. This is what would be expected from our present knowledge of the tonicity of the accelerators. An examination of the pulse rates recorded in Hering's experiments given in Table E, page 469, shows that in every case where the normal resting rate was given it became higher after the operation. In five of the seven experiments it remained higher, and in three cases the rate kept increasing following the recovery of the animal. The motor acceleration following the operation was at first small but it gradually increased.

The possibility was suggested by Hering that in tearing out a part of the sympathetic the function of the remaining portion might be injured, so that in the first exercise experiments the sympathetic accelerator nerves would be partly anatomically and partly physiologically removed from activity. On the return of function to the injured nerves the motor acceleration of the heart would, of course, increase. This possibility he could not

eliminate with certainty. In his method of operation the sympathetic which runs separate from the vagus in the rabbit was followed down to the first thoracic ganglion, which was secured and torn off from the sympathetic chain. The injury was variable in the different cases, in many of which he tore out three ganglia. While post-mortem was made in most cases, he believed the findings were of doubtful value as the field was so scarred over it that it was difficult to find any remaining branches from the sympathetic to the heart.

While Hering's explanation may in a measure be correct it is more probable, inasmuch as the heart was much more rapid after the operation, that the fault lay in an impairment of the vagal action either centrally or peripherally. The latter was probably the reason, because in Hering's description of his operation he says that the nerves which were connected with the ganglion, the depressor and branches of the vagus, were cut across. Furthermore, Friedenthal and Schaternikoff[12] state that in performing this operation a portion of the cardio-inhibitory fibres are always removed.

On the basis of injury to the vagi the failure of Hering's rabbits to accelerate is readily explained. When the heart is not under normal vagus control an increase in rate can hardly be secured by inhibition of the vagal centre. Thus in experiment No. 23 (1) (Table E) where the resting rate was high after the operation and kept on increasing during the succeeding days, the motor acceleration fell far below the normal, and on subsequent section of the vagi the pulse rate fell in spite of the fact that Hering had previously shown that there is considerable vagus tone in the rabbit. On the other hand in experiments No. 12 (II) and No. 14 (II) where the pulse rate fell below the normal resting rate on recovery from the operation, the motor acceleration reached that attained by the intact animal and in No. 14 (II) in which subsequent vagotomy was performed the pulse rate rose following the operation.

A criticism of our results may lie in the possibility of accelerator fibres being present in the vago-sympathetic trunk. However, the improbability of such fibres having any great importance was shown by Hunt. He found that evidence of accelerator

fibres in the vago-sympathetic of the dog occurred only in exceptional cases and when it did occur the acceleration produced was not at all like that obtained reflexly.

The fact that acceleration at the beginning of exercise persists after removal of the accelerators would in itself seem to prove that the increased rate was brought about by means of the inhibitory mechanism. That this is true we have tried to establish in several ways.

As already pointed out the extremely short latent period for exercise acceleration in the normal animal seems explicable to us only on the basis of nervous mechanisms being involved. If this be true, the mere fact that acceleration persists after removal of the stellate ganglia is sufficient proof that the efferent path to the heart is by way of the vagi, provided that the acceleration is of the same type as normally.

If the accelerations during exercise before and after the removal of the stellate ganglia be compared in percentages of the resting rate, it will be found that the acceleration in every case but one is greater after removal of the ganglia. This results from the fact that although the resting rate falls after removal of the accelerators the power to increase the rate a given number of times remains about the same as before. Cardiac acceleration in the normal animal as expressed in percentages is thus largely dependent on the resting rate, or what is the same thing, accelerator tone. In the six intact animals the percentage accelerations for 2 minutes exercise varied from 31 per cent to 66 per cent, averaging 43 per cent. After removal of the accelerators the same amount of exercise produced increases varying from 55 to 72 per cent with an average of 61 per cent. These percentages are of little importance, aside from the fact that they show how little the power of the heart to accelerate in exercise depends on the accelerator mechanism.

Our protocols show that acceleration occurred as quickly after the removal of the accelerators as before. This is in contrast to the results which will be given later of experiments in which the vagi alone were sectioned. Furthermore, the actual acceleration remained approximately the same after the stellate ganglia were removed. These observations seem clearly to indicate that the

same mechanism was active in both cases, that is, the inhibitory mechanism. Bowen[3] and Aulo[10] have each shown that acceleration produced by depression of the vagal centre has the same characteristics as that seen in the early stages of exercise. In each the increased rate is brought about by the shortening of the diastole.

By the use of atropine we attempted to determine whether depression of the inhibitory mechanism was alone sufficient to account for the accelerations observed. In dogs Nos. 1, 2, and 3 after 2 mg. of atropine injected subcutaneously the heart rates rose to 232, 230, and 188 per minute respectively. The rates after 2 minutes exercise were 160, 148, and 184. In these same animals after removal of the stellate ganglia 2 mg. of atropine caused the heart to rise to 206, 168, and 200 respectively. The data show that whether the accelerators are intact or not the heart still has a certain power of acceleration which is greater by a large margin than that needed during the early stages of exercise. Diminution of vagal tone can, therefore, readily account for the exercise accelerations.

The question as to whether the inhibitory mechanism was still responsible for the accelerations occurring after extirpation of the accelerators seemed capable of being put to a crucial test by the simple expedient of cutting the vagi. At intervals varying from 1 to 18 weeks after removal of the stellate ganglia the six dogs were therefore vagotomized. The data obtained after this procedure will be presented in the next section.

II. Effects of Vagotomy after Previous Removal of the Stellate Ganglia

1. The resting pulse. — After vagotomy in dogs whose accelerators had previously been cut, the pulse rate was greatly increased, its highest point being reached immediately after the operation. Three of the dogs were kept longer than two days after the operation and in these it was noted that the pulse rate fell rapidly from this maximum. Thus in dog No. 6, with a resting pulse of 72 after ganglicctomy, the rate went up to 160 immediately after vagotomy. Twenty-four hours following, the

pulse rate was 120, 36 hours following it was 116, at which figure it remained until the end of the third day when the animal was killed.

This fall in rate occurred no matter whether the vagi were cut previous to or subsequent to removal of the accelerators. Thus in dog No. 9, in which the accelerators were intact, immediately after section of the vagi the pulse rate rose to 200 per minute, falling on the second day to 162 per minute, and on the third day to 154 per minute. In both cases this drop in pulse rate was probably due to an impairment of the heart's automaticity by its unrestrained activity.

Our protocols show the resting rates after vagotomy to be from 42 to 96 beats per minute faster than after the previous removal of the accelerators. The wide differences in these figures is largely due to the differences in time of the observations after vagotomy. They all show the existence of a marked vagal tone: The figures all fall short of the rates observed when the vagal endings were blocked by atropine after removal of the accelerators, but this is to be expected since the latter observations were made immediately after the administration of the atropine while the automaticity of the heart was still at its maximum.

2. The reaction to muscular exercise. — Much to our surprise the dogs showed a marked acceleration on exercise after all the extrinsic cardiac nerves were cut. These accelerations varied somewhat in different animals, and differed greatly in the same animal on different days. The accelerations on the first day, a few hours after the vagotomy, were very high, but by the end of the second day the accelerations due to the same amount of exercise had become greatly reduced. Thus dog No. 4 increased his pulse rate 64 beats per minute on the day of vagotomy, but on the day following, the same amount of running gave an acceleration of only 18 beats. Similarly dog No. 6 accelerated 68 beats per minute at first and on the next day only 12 beats. The time of exercise was reduced from two minutes to thirty seconds in these experiments on account of the marked cyanosis and vomiting which were present immediately following vagotomy. It will be noted that the accelerations immediately following vagotomy were greater than those observed before any of the

nervous mechanisms were removed. Results similar to these were obtained on four of the six dogs. The data on two of the dogs were incomplete since our attention had not yet been called to this point.

The fact that the accelerations immediately following vagotomy were at times higher than in the intact animal suggested to us that possibly an entirely new factor had been introduced. It was observed that the dogs exercised on the same day that the vagotomy had been performed became extremely cyanotic, due to the slow respiratory rate and the almost entire inability of the respiratory organs to meet the demands of the moment. On the day following the operation, however, the respiratory exchange seemed to be sufficiently adequate to prevent any marked cyanosis during the same amount of exercise. Just how this improvement was brought about we are not entirely clear. In part it seemed to consist of a slight permanent increase in rate and the ability to accelerate a few respirations per minute during the exercise. Such an acceleration does not necessarily conflict with the conclusions of Scott,[13] who showed that after vagotomy the rate of respiratory discharge could not be raised by increasing the carbon dioxide content of the blood. Psychic impulses or even temperature of the blood might have had an effect in our animals. Stewart and Pike[11] cite the case of a dog in which on emotional excitement the respiratory rate increased after vagotomy. Whatever the means of adaptation may have been in our animals the same amount of exercise on the second or third day following vagotomy gave very few of the distressing cyanotic symptoms observed during the same procedure shortly after the operation. Along with the disappearance of the cyanosis, retching and vomiting, the exercise acceleration showed its marked decrease.

It seemed, therefore, that the great acceleration occurring at first might be due to the asphyxia. That this was the case we proved on dog No. 6. On the day following vagotomy when the pulse rate could only be increased 12 beats by the same exercise that on the day before had given an increase of 48, the dog was asphxyiated for 30 seconds. This was done by wrapping a wet towel around his head. The struggling during this procedure was probably no more than equivalent to the exercise previously

given, but the pulse rose to 208 per minute, thus almost attaining the frequency observed when exercise was given immediately after vagotomy. Evidently the marked accelerations observed after vagotomy were in some way associated with asphyxia.

The recent work of Von Anrep,[14] who showed that there is a secretion of the adrenals during asphyxia arising through stimulation of the centres of their secretory nerves, suggested a possible explanation of this asphyxial acceleration. The hypothesis could readily be put to a crucial test. When dog No. 6 was made to exercise for thirty seconds by struggling during asphyxia its heart accelerated 92 beats per minute. If ligation of the blood vessels to the adrenal glands should prevent this acceleration, the conclusion would be justified that the acceleration was due to a secretion of the adrenals. This experiment was therefore performed. The blood vessels were tied under ether anaesthesia. The animal was allowed to recover, and exercise during asphyxia was tried one and one-half hours after the operation, before the intervention of the marked asthenia which follows removal of the adrenals in dogs. The marked acceleration was now absent. Asphyxia and struggling for 90 seconds produced an acceleration of only eight beats per minute. This acceleration may be attributed to temperature and it shows how relatively small a role that factor plays. That this lack of acceleration was due to removal of the adrenals and not to depression following the operation, was shown by a similar experiment on a dog whose extrinsic cardiac nerves were intact. In this case although the resting pulse was 224 per minute and the dog had become very asthenic and would exercise but little, the heart still accelerated to 248 beats per minute.

The marked acceleration which was observed during exercise immediately after all the extrinsic cardiac nerves were cut was therefore due largely to a secretion of the adrenals. As shown by Eyster and Meek,[15] the action of the adrenalin is independent of the integrity of the accelerator nerves. The acceleration found on the second and third day after the vagotomy, when the signs of asphyxia were reduced or absent, remains to be explained. The greatly reduced power to accelerate at this time indicates clearly the importance of the role which the inhibitory mechanism

had been playing. Gangliectomized dogs that accelerated on an average of 41 beats per minute after two minutes exercise were able after a subsequent vagotomy to accelerate only 16 beats per minute, provided there were no signs of asphyxia. The inhibitory mechanism was then responsible for the major part of the acceleration observed after extirpation of the accelerators.

To explain the limited acceleration possible after the signs of asphyxia had passed off, four possibilities suggest themselves, namely: changes in blood pressure, respiratory rate, temperature, or composition of the blood.

A review of the literature shows quite clearly that the blood pressure has no effect on the isolated mammalian heart, and that in those experiments which seem to show the contrary the temperature was not controlled. It was first shown by Martin[16] that between the pressure limits of 30 and 150 millimetres of mercury the pulse rate is not in the least influenced, provided the composition and the temperature of the perfused blood were kept constant. The recent work of Knowlton and Starling[17] confirms this result.

In a large percentage of animals whose hearts are normally slowed by tonic activity of the vagi, such as the dog, there is an acceleration of the pulse which occurs synchronously with inspiration. It would be expected, therefore, that when the respiratory rate increased an acceleration of the pulse rate would result. This could hardly explain the increase in our experiments, however, for the simple reason that the increase in respiratory rate after vagotomy was at most very little and in several cases showing the usual slight acceleration it did not occur at all.

However, to rule out completely any mechanical or nervous effects on pulse rate arising from vagal breathing simultaneous records of the respiration and heart beat were made by means of a pneumograph and the string galvanometer. The speed of the records was made such that measurements to .01 second were possible. No differences occurring synchronously with changes in respiratory phase could be discovered. A further investigation was then made of the lengths of the heart cycles when the respiration was increased in rate and depth. For this purpose 2.5 c.c. of M/50 NaCn. was injected in the ear vein of

the unanaesthetized animal. The amplitude of respiration was much increased and the rate rose from 5 to 12 per minute. In spite of these increases no effect was observed in any stage on the length of the cardiac cycle.

Of the two products of muscular activity which could produce changes in other parts of the body by altering the condition of the blood, heat and metabolic products, attention was paid only to the latter in the earlier work on exercise. Johannson[7] attributed the comparatively small accelerations that he obtained when the hind limbs were tetanized by stimulation of the lumbar cord to fatigue products, but furnished no proof for this assumption. Hering,[6] whose paper appeared shortly after that of Johannson, suggested that this might be the cause of the accelerations he obtained after the inhibitory and accelerator nerves to the heart were cut. The experiments of Johannson were confirmed by Athanasiu and Carvallo,[2] who, however, present no additional evidence that products of metabolism were the real cause. It has, however, been rather generally accepted that at least in prolonged exercise the effect of fatigue products directly on the heart is added to the acceleration which has already resulted through the nervous system.

Considerable doubt has been thrown on this view by recent work. Mansfeld found that intravenous injection of the extract of fatigued muscle produced no more cardiac acceleration than a corresponding amount of the extract of normal muscle. We have already mentioned the work of Petersen and Gasser, who found that while substances are formed in the fatigued muscle which affect the size of the beat, these substances are without effect on the rate. We must, therefore, next consider the temperature change produced by exercise as a possible explanation of the acceleration in pulse rate occurring after all nervous connections are severed.

While it has been known since the experiments of Newell Martin that the rate of the isolated heart increases as the temperature rises and decreases as it falls, little attention has been paid to it in the problem of the acceleration during exercise. This is probably largely due to the fact that it has been found that the temperature curve does not closely parallel the curve of heart

rate. It is by the work of Mansfeld[5] that interest has recently been revived in temperature changes. However, according to his view, the action of heat is not expressed merely in the increased chemical activity of the heat, but the change in temperature stimulates the endings of afferent vagal nerve fibres in the heart which produce a reflex stimulation of the accelerator centre. This conclusion is based on the fact that when the hind limbs of his animals were isolated from the central nervous system and tetanized, he obtained marked accelerations if the temperature of the animal was kept above 36 degrees. These did not occur when the reflex arc was cut.

Application of this theory to our observations shows how far it falls short of explaining the facts. In the first place the accelerations in our animals were as good after the removal of the stellate ganglia as before, which according to Mansfeld's theory, would be impossible since the efferent neurone of the arc he describes was interrupted. In the second place, the heart has considerable ability to accelerate still left after both the accelerator and inhibitory nerves are cut, which, if due to the rise of temperature, must be caused by a direct action.

The rise of temperature in our experiments observed after the two-minute period of exercise was in most cases .4° to .5° C. In the isolated heart Martin found that for temperatures between 37° C. and 40° C. a variation of one degree in the perfusion fluid changed the heart rate from 5 to 18 beats per minute. The figures published by Knowlton and Starling gave increases of 3 to 12 beats per minute for similar conditions. Increased temperature of the blood may be somewhat more effective in the intact animal than in case of the isolated heart. If a slight advantage of this kind is granted the accelerations we have obtained after removal of all nervous connections and after asphyxial effects have abated is quite sufficient to explain the residual acceleration, averaging 16 beats per minute, which we have observed.

III. Effects of Exercise after Cutting the Vagi Alone

An attempt was made to study in a positive way the part played by the accelerators in the early acceleration of exercise.

To do this dogs were vagotomized and a day later the adrenals were ligated. As was to be expected, exercise immediately after the vagotomy produced cyanosis and vomiting with a very rapid heart rate. In one case, dog No. 10, the pulse increased from a resting rate of 160 per minute after vagotomy to 224 on exercise. From our previous experience it seemed impossible to tell whether this increase was due to an asphyxial secretion of adrenalin or to a stimulation of the accelerator mechanism. This point we tried to settle by tying off the adrenals and again exercising.

Two hours after extirpation of the adrenals while the dog was in good condition, the same amount of exercise increased the pulse from a resting rate of 160 to 184. It was noted that the maximum increase occurred in the third and fourth quarter minutes after a 30-second period of exercise, which was of course typical of accelerator stimulation. The increased rate after removal of the adrenals was about one-half that observed in the normal animal. A second experiment verified the findings just mentioned, the acceleration after removal of the adrenals being, however, somewhat less. The loss of the inhibitory mechanism seems, therefore, to interfere more with exercise acceleration than does the loss of the accelerators. The definite acceleration after section of the vagi and extirpation of the adrenals shows, however, that although the accelerators may not play a leading part in the intact animal, yet it is possible through them to cause an increase in pulse rate.

IV. Discussion

Our work has led us to believe that the heart as a result of exercise may under certain conditions be accelerated in at least four different ways; namely, by a decrease in vagal tone, by stimulation of the accelerators, by a secretion of adrenalin and by an increase in the temperature of the blood. Just what part does each of these factors play in the increased pulse rate following ordinary voluntary exercise? Before attempting to answer this question one should have clearly in mind that there are apparently two types of acceleration following exercise, one immediate and the other prolonged. Our experiments and our conclusions apply only to the immediate response of the heart.

The cause of the increased heart rates, which may persist for long periods after exercise has ceased, seems to us to be a separate and distinct problem.

The rapidity with which acceleration of the heart rate follows exercise as shown by Bowen, Buchanan and ourselves, the vagal character of the acceleration as pointed out by Hunt and Aulo, and the fact shown by our work that the reaction of the heart to exercise is impaired by removal of the inhibitory mechanism but practically unaffected by extirpation of the accelerators, all seem to give ample proof that the first acceleration during exercise in the normal animal is produced by inhibition of vagal tone. Our data give no evidence as to whether this inhibition is brought about by an irradiation of motor impulses in the medulla according to Johannson, or whether it is a reflex from peripheral muscular end organs according to Athanasiu and Carvallo. The marked acceleration found after extirpation of the stellate ganglia does, however, invalidate any explanation depending on the accelerators as part of the reflex arc.

Inhibition of the tone of the vagus is the most economical means by which acceleration of the heart can be brought about. On account of the great differences in rate demanded of the heart in different states of bodily activity, the potential automaticity of the heart is high but is held in check by the central nervous system through the vagal inhibitory mechanism. This potential automaticity, which may be defined as the maximal rate of impulse formation when the heart is free from all control, is more than sufficient to allow for the accelerations occurring during exercise. By releasing the check on this automaticity, the acceleration in rate demanded by increased activity can readily be produced. An analogy may be drawn with a battery circuit in which the amount of current is controlled by a resistance box. If an increased current is demanded which is still less than the battery is able to produce, the most logical procedure would be to remove some of the resistance rather than to add more batteries.

The accelerator mechanism might be regarded as a factor of safety superimposed on the vagus to meet the stress of extreme conditions. The fact that it can produce a certain increase in

heart rate after section of the vagi and ligation of the adrenals would be in accordance with such a view. It was brought out in Hunt's work that the accelerator mechanism is very resistant to pathological conditions such as low blood pressure, asphyxia, and drugs such as curare and the anaesthetics. The recent work of Kuntz [18] on the development of the sympathetic nervous system in vertebrates is of interest in this connction. He found that the vagal sympathetic plexuses were the first to arise in the course of evolution and only as specialization advanced was any part in the nervous control of the internal functions shifted posteriorly. If this be true it follows that the accelerator mechanism is added to the phylogenetically older inhibitory mechanism.

The part played by temperature in the acceleration immediately following exercise must be small. For this factor to affect the heart a comparatively long latent period is necessary. When, however, the blood of the body finally reaches a higher temperature, it must have its effect on the heart rate. This influence may be important in prolonged exercise but ordinarily it is added to the vagal effect and thus obscured. Our experiments show that accelerations from this cause could not exceed about 16 beats per minute.

The amount of acceleration that is due to the secretion of adrenalin in the intact animal is probably negligible. As is well known, when the vagi are intact the injection of adrenalin is followed by a slowing of the heart due to action either directly or reflexly on the cardio-inhibitory centre. Exercise with dyspnoea would doubtless call forth a secretion of adrenalin in the normal animal, but so far as we know with the vagi intact this would not express itself in an increase of heart rate. It might, however, be useful and important in augmenting the strength of the beat. In this way the heart might be benefited by the secretion of adrenalin which Cannon and de la Paz [19] have shown to occur at times of great emotion. That exercise as such has little effect on the adrenals has already been shown in our experiments. After the asphyxia succeeding vagotomy had somewhat abated, the same amount of exercise no longer greatly accelerated the heart.

Summary

Acceleration of the heart at the beginning of voluntary exercise in the normal animal is chiefly due to the decrease in tone of the cardio-inhibitory centre. Our evidence for this conclusion is the following: (1) Electro-cardiograph records confirm the work of Bowen and Buchanan that acceleration takes place as early as the cardiac cycle following the initiation of the exercise. (2) Acceleration of the heart at the beginning of exercise persists after the removal of the accelerator mechanism. The actual increase in number of beats due to a given amount of exercise has been found to be practically the same in six dogs before and after removal of the stellate ganglia. One of these animals was observed for over four months. (3) Acceleration on exercise is reduced after section of the vagi provided that all asphyxial effects are excluded.

After the removal of the accelerators and subsequent section of the vagi marked acceleration of the heart may still be produced by a short period of exercise. This is associated with the cyanosis following vagotomy and on the second day when the animal is able to do the same work without such a marked cyanosis the increase following exercise is greatly reduced. It may be increased again by asphyxiating the dog for some 30 seconds. After tying off the adrenals neither asphyxia nor exercise gave a marked increase in heart rate. Exercise involving asphyxial conditions may then be accompanied by a secretion of the adrenals. In the normal animal with the vagi intact this secretion of adrenalin can hardly be supposed to affect the heart rate. It may, however, cause an increase in the amplitude and force of contraction.

In all six dogs the heart rate after removal of the accelerators was found to be remarkably constant, averaging about 72 beats per minute. The resting pulse rate of each animal is therefore believed to depend more on accelerator tone than on any other factor.

After removal of all nervous control and elimination of the adrenals exercise may still cause a small acceleration of the heart. This is attributed to the increased temperature of the blood.

Acceleration of the heart occurs on exercise after vagotomy

and extirpation of the adrenals. The amount though small is more than can be accounted for by increased temperature of the blood. Acceleration may therefore be brought about through the accelerators if necessary. Our work leads us to believe that the accelerators are a factor of safety and that in exercise their action is superimposed on that of the vagi only in times of great need. Aside from this their chief function is maintaining the level of the resting pulse.

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THE NERVE CONTROL OF THE THYROID GLAND[1]

By JESSIE MOORE RAHE, JOHN ROGERS, G. G. FAWCETT, AND S. P. BEEBE

[*From the Department of Experimental Therapeutics, Loomis Laboratory, Cornell University Medical School, New York City*]

TWO mechanisms are established within the animal body by means of which the functions of its different parts are co-ordinated. One of these acts through the medium of the nervous system, while the other is chemical in nature. The two are, however, interdependent to some degree. The activities of the nervous system in this respect have long been a subject for accurate investigation, but the work of recent years has served to emphasize the importance of the hormones, the chemical messengers by means of which widely separated structures are brought into a harmony of action. At some link in the chain it will be found, however, that the determining influence of the nervous system is predominant.

The thyroid gland is a favorite example of the glands which produce internal secretions. Its functions and pathological conditions are perhaps better known than any others in its class. Variations in its functions are correlated with fairly well-defined clinical conditions. A large number of studies have been made upon the chemical nature of its active substance, and many attempts have been made to show how its activity is regulated, at least in part, by the secretions of other ductless glands.

It seems very probable that the active substance in the thyroid gland contains iodine in combination with protein, and that the physiological activity is in proportion to the quantity of iodine in the combination. We believe that the function of this substance is not performed within the gland itself. The colloid of the gland may be looked upon as reserve material ready for dis-

[1] The expenses of this research were defrayed by the Johnston Livingston Fund for Experimental Therapeutics.

charge into the blood or lymph, through which medium the general tissues of the body are supplied. Some attempts have been made to demonstrate the presence of this active substance in the blood by means of a biological reaction, but none of them have thus far been successful. If one considers how very vascular the gland is, and how small a quantity of the active substance is distributed through the blood leaving the gland in twenty-four hours, it is not surprising that none of these methods have satisfactorily demonstrated the presence of the iodized protein in the circulation.

Recently Asher and Flack[1] have attempted to demonstrate the nerve control of the thyroid. Their experiments are indirect and are so complicated as to leave the matter in great doubt. Their experiments were made upon rabbits, cats and dogs, with a view to determine whether any phenomena could be observed after stimulation of the thyroid nerves which would lead to the conclusion that stimulation had caused an increase in thyroid secretion in the blood. As a means to this end they compared the 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 otherwise exactly similar experimental conditions, a stimulation of the depressor nerve or an intravenous injection of adrenalin was more effectual during a stimulation of the thyroid nerves than shortly before without it. That both these symptoms, viz., increased depressor excitability and more effective action of adrenalin, actually depend upon an inner secretion of the thyroid and not upon any accompanying circumstance of nerve stimulation is proven by the fact that on extirpation of the thyroid the symptoms no longer appeared, and on the other hand intravenous injections of thyroid extracts acted exactly like stimulation of the thyroid nerves. Their experiments are complicated and while suggestive they do not satisfy.

The purpose of the experiments reported in this communication is to give evidence of a different character that the thyroid gland is, at least in part, under nerve control. Briefly, the plan of the experiments was to determine, first, whether the iodine content of the two lobes of the normal thyroid gland from the

same animal is identical; and second, whether stimulation of the nerves leading to one lobe causes a loss in its iodine content. If the two lobes of the normal thyroid are identical in iodine content they may serve as controls of one another. Granting such to be the case it was our hypothesis that stimulation of the nerves to one lobe might cause a change in the iodine content because of the resulting discharge of iodized proteid from the gland.

The method followed in determining the iodine in all the analyses herein reported was the modification of the Baumann process devised in this laboratory by Riggs.[2] Some very slight changes in the technique were made which experience has shown to be valuable in rendering the fusion easier and in lessening the tendency to form iodates to such a degree that many of the fusions were wholly free from such compounds. The procedure in detail was as follows: the fresh glands were carefully trimmed from any adhering tissues and accurately weighed; they were then placed in a nickel crucible and covered with 10 to 15 c.c. of a 50 per cent solution of sodium hydroxide. Heat was carefully applied and the glands disintegrated and finally fused. Such a technique is preferable to the use of the solid hydroxide in that it gives a gradual disintegration of the gland and allows carbonization to take place at a lower temperature. At no time was more than a dull red heat applied and often not that. When the mass had been thoroughly fused, only as much sodium nitrate was slowly added as was necessary to oxydize any small particles of carbonaceous material remaining. After cooling, the fused mass was dissolved with hot water, quantitatively filtered, made up to exactly 100 c.c. and thoroughly mixed. From this point the method of Riggs was followed exactly.

In this laboratory we have found that in determining such small amounts of iodine as is found in dogs' thyroids the method is preferable to that of Hunter,[3] in that the fusion is more easily carried out, and it requires the use of fewer standardized reagents. In Kendall's[4] method the fusion is quite similar except that he uses larger amounts of sodium hydroxide and potassium nitrate, and reduces excess by the use of gallic acid which in itself causes trouble if too much is added. His method for titration after usion is more complicated than Riggs' colorimetric method and,

in this laboratory, has not been found more accurate when using minute quantities of iodine.

For the purpose of determining whether the iodine content of the two lobes of the thyroid is the same per gram of fresh gland several animals were killed and the glands immediately dissected out and subjected to the method outlined above. No particular selection was followed with reference to the animals, and in no case was there found any gross pathological condition of the gland. They were the usual animals kept in stock and had been fed the same diet which we have used for a number of years, viz. boiled beef hearts and bread.

Table I shows the results of these analyses:

TABLE I

SHOWING IODINE CONTENT OF THE TWO SEPARATE THYROID LOBES FROM THE SAME ANIMAL

Lobe	Weight of lobe	Milligrams Iodine found	Milligrams Iodine per gram of gland
R	0.763	0.300	0.393
L	0.886	0.350	0.395
R	0.7048	0.475	0.674
L	0.6600	0.450	0.681
R	0.7174	0.700	0.975
L	0.7620	0.750	0.984
R	1.2786	0.900	0.7038
L	1.1400	0.800	0.7011
R	1.0956	1.100	1.004
L	0.9872	1.000	1.012
R	0.9810	1.440	1.468
L	0.8154	1.200	1.472
R	0.3602	0.132	0.3664
L	0.4020	0.145	0.3607

From the table given it is evident that the iodine content varies widely in the different animals, but the two lobes are wonderfully constant, the difference shown falling within the limit of error of the analytical method. The largest difference

noted is found in the third pair of glands, in which there is a difference of only .009 mgms. per gram of fresh gland.

The figures are based upon the weight of the fresh gland rather than of the dried substance, because most analyses of the iodine content of these glands recorded in the literature are so expressed, and furthermore because it is probably more accurate. It is not a simple matter to dry the glands to constant weight and transfer the dried powder without loss to a fusion crucible, and the fusion of the moist gland in the manner described above is much simpler.

It seems evident from the analysis quoted that one lobe of the thyroid may serve as control of the other provided both lobes are normal.

The method of conducting the stimulation experiments was as follows: The stimulation in each instance was electrical and was obtained by connecting three dry cells in series for the primary current. This current was connected with the ratchet wheel of a clock in such a manner as to permit a momentary stimulus every ten seconds. The current from the secondary coil was so regulated that only a faint stimulus was given to the moist tongue of the operator.

The nerve supply to the thyroid probably has its origin in filaments from the sympathetic. In man a nerve filament can be demonstrated to arise from the superior cervical ganglion of the sympathetic, and to follow more or less closely the course of the superior thyroid artery, and to terminate in the upper anterior portion of the superior pole of the thyroid. In addition there are microscopic filaments from the sympathetic which can be demonstrated to enter the gland in the walls of both the superior and inferior thyroid arteries and sympathetic filaments are apparent around each of the thyroid vesicles.[5]

In the dog the same nerve filament exists entering each of the separate thyroid lobes near the outer superior pole, but the cervical sympathetic and the pneumogastric nerves form one strand and the apparent nerve supply of the gland cannot be proved by dissection, as in man and the cat, to arise only from the sympathetic. Nevertheless it is possible that the dog's anatomy is not different from that of the cat and man in this respect.

In these experiments ether anaesthesia was used in each case and was administered through a bottle with a cannula in the trachea. The method of arranging the stimulation was varied in the different experiments.

Stimulation Experiments — Series I.

In these experiments one lobe of the gland was removed immediately before the stimulation was begun. This lobe served as a control. The vessels of the upper pole of the remaining lobe were carefully separated from the connective tissues and the electrodes were then carefully brought into contact with the vessels and the accompanying nerve filaments. Careful approximation of the electrodes was carried out so that the blood supply and return would not be impaired.

(In the fifth experiment in this series both lobes were left in until the end of the period of stimulation.)

TABLE II

Showing Iodine Per Gram of Control and Stimulated Gland. Series I.

Gland	Wt. of gland grams	Iodine in mgms. per gram	Length of stim.
Control	.485	.2051	2 hrs., 30 m.
Stim.	.417	.1923	
Control	.4062	1.1078	3 hrs.
Stim.	.3717	1.0088	
Control	1.0680	.8895	3 hrs.
Stim.	1.1234	.7566	
Control	1.2280	1.3029	3 hrs.
Stim.	1.2000	1.0417	
Control	.6564	.9012	2 hrs., 30 m.
Stim.	.5770	.7312	

The average loss in iodine was .1351 mgms. per gram of gland, but in that case No. 4, in which the odine content of the control gland was highest, the loss on stimulation was .2612 mgm. iodine per gram of gland, a loss of approximately 20 per cent. In every

stimulated gland there has been a loss so great as to be beyond the possibility of error in the determination.

Stimulation Experiments — Series II

In these experiments both lobes remained intact throughout the experiment. After the period of stimulation they were both removed and prepared for analysis. The point of stimulation was on the combined vagus and sympathetic. The vagus on the side stimulated was ligated low down on the neck, a careful dissection was made, the superior ganglion exposed, and the nerve cut central to the ganglion. The electrodes were applied to the nerve peripherally to the ganglion. The exposed tissues were covered with cotton moistened with Locke's solution.

TABLE III

Showing Iodine in Milligrams per Gram of Gland after Stimulation Through Nerve

Gland	Wt. of lobe in grams	Iodine in mgms. per gram of gland	Time of Stim.
Control	2.7573	.1813	2 hrs., 30 m.
Stim.	2.5273	.1582	
Control	.6174	.3239	3 hrs.
Stim.	.8126	.1723	
Control	1.0646	1.3620	2 hrs., 15 m.
Stim.	1.2070	1.0270	

The analytical results in this series show a larger percentage loss of iodine than in those quoted in the previous experiment. In the second animal nearly 50 per cent of the iodine in the stimulated lobe was discharged into the blood or lymph during the period of stimulation.

Stimulation Experiments — Series III

In this series the thyroids were both left intact until the termination of the experiment. The combined vagus and sympathetic were isolated about one inch below the thyroid cartilage

and the stimulation which was identical with that used in the previous experiments was applied at this point. The nerve was not cut or ligated in any manner. The circulation through the gland was not impaired or disturbed by the operative procedure.

TABLE IV

Showing Loss of Iodine in Glands Stimulated Through the Intact Nerve

Lobe of gland	Weight in grains	Iodine in mgms. per gram	Loss	Time
Control	.9469	.581		45 min.
Stim.	.8048	.559	.022	
Control	2.8732	2.866		3 hrs.
Stim.	3.1000	1.967	.899	
Control	.5228	2.486		3 hrs.
Stim.	.5008	2.196	.290	
Control	.7450	2.013		3 hrs.
Stim.	.5425	1.843	.170	
Control	.4450	.272		30 min.
Stim.	.6660	.241	.031	
Control	.4738	2.216		3 hrs.
Stim.	.6647	2.031	.185	
Control	.7830	1.277		3¼ hrs.
Stim.	.8730	1.027	.250	

Summary

The experimental results may be summarized in the following way:

1. The average difference of iodine, expressed in mgms. per gram of fresh gland, between the two thyroid lobes of seven normal dogs was .0055 mgms.

2. The average difference found when the superior vessels, with accompanying nerve fibres, of one lobe were stimulated was .1351 mgms.

3. The average difference found when the stimulus was applied to the vagus peripherally to the superior ganglion, the nerve being cut central to the ganglion and ligated peripherally to the stimulus, was .1699 mgms.

4. The average difference found when stimulus was applied to the intact vagus was .2640 mgms. In series III two glands were stimulated 45 minutes and 30 minutes respectively. If the loss in the remaining five glands which were stimulated three hours is averaged it will be found that a loss of .3588 milligrams per gram of gland occurred.

In no case did we fail to get a loss after stimulation. There seems to be no reasonable doubt from these results that the thyroid is at least in part under nerve control, and that its physiologically active substance is discharged into the circulation in response to a nerve stimulus.

[1] Asher and Flack: Zeitschrift für Biologie, 1911, lv, p. 83.

[2] Riggs: Journal of the American Chemical Society, 1910, xxxii, p. 692.

[3] Hunter: Journal of Biological Chemistry, 1910, vii, p. 321.

[4] Kendall: Journal of the American Chemical Society, 1912, xxxiv, p. 894.

[5] Rhinehart: American Journal of Anatomy, 1912, xiii, p. 91.

THE VARIABILITY OF BLOOD PRESSURE AND OF VASOMOTOR IRRITABILITY IN THE ANAESTHETIZED DOG

By R. G. HOSKINS and HOMER WHEELON

[*From the Laboratory of Physiology of the Northwestern University Medical School*]

IN connection with other researches we have found it necessary to determine somewhat definitely the extent of variation of blood pressure and of vasomotor irritability at different times in the individual anaesthetized dog, under ordinary laboratory conditions. The matter seems to have received little exact investigation. Pawlow,[1] however, has reported an instance of a dog in which an arterial cannula was left for a number of days. He made several blood pressure determinations, but without anaesthesia. The pressure at different times proved remarkably constant. A series of readings during twenty-one days showed pressures of 128, 131, 128, 129, 131 millimetres of mercury. As regards changes of vasomotor irritability some data have been secured in the use of the blood-pressure method of assaying epinephrin. Under the conditions of any given experiment there is a considerable degree of constancy in the reactions to this drug. The literature on the subject has been reviewed by Schultz.[2] We are not aware, however, of any previous determinations in the same animal at different times.

Our experiments were made upon medium sized dogs, about 10 kilos in weight, with the exception of one which weighed 17 kilos. Males or females were taken at random. Two or three blood-pressure determinations were made on each animal. An interval of five to ten days between successive determinations

[1] Pawlow: Archiv für die gesammte Physiologie, 1879, xx, p. 216.

[2] Schultz: Bulletin No. 61 Hygienic laboratory, public health and marine hospital service of the United States, 1910.

permitted recovery from the preceding operation. As Poiseuille [1] showed nearly a century ago there is little difference in mean pressures in the larger arteries, consequently any of these could have been used. Owing to their accessibility, however, we used the right and left femorals and a carotid. Blood pressure was recorded by means of an ordinary mercury manometer and float.

Ether administered by the open cone method was used throughout the series. It is of course possible by varying the depth of anaesthesia to produce marked differences in arterial pressure. On the other hand, if the anaesthetic is carefully administered in amount just sufficient to keep the corneal reflex in abeyance, a long-continued record can be secured in which the pressure is practically constant. It was at this depth of anaesthesia we aimed to work. In the earlier experiments each dog was given an injection of morphine, 0.1 to 0.2 grain, the amount being constant for any given animal. It was found, however, that this procedure added little to the smoothness of the anaesthesia and it was discontinued.

To permit a series of observations in the same animal aseptic precautions were observed in making the determinations. In securing an aseptic technique the use of a Hall reservoir cannula proved fortunate. This cannula was devised by Dr. W. S. Hall to obviate the necessity of a pressure flushing system. It consists of an ordinary glass arterial cannula in which is blown a bulb of about 20 cc. capacity. This is filled with anti-coagulating solution and connected directly with the manometer. To prevent too rapid diffusion into the artery but at the same time keep the fluid supplied to the blood in sufficient amount, the part of the cannula below the bulb should be conical in shape and the lumen of the apex comparatively small. With reasonable expedition in carrying out the experiments little trouble with clotting is experienced. In the earlier observations half-saturated solution of sodium carbonate was used as anti-coagulent. A single charging with this prevented coagulation for an hour or longer. Direct experiments showed that no immediate deleterious results followed; the post-operative mortality rate, however, was high. Substitutions

[1] Poiseuille: Thèse de Paris, 1828. Cit. by Tigerstedt, Physiologie des Kreislaufes, Leipsig, 1893, p. 351.

of 5 per cent solution of sodium citrate greatly lowered this rate without seriously impairing the efficiency of the method. Occasionally, however, a cannula had to be removed and recharged.

All necessary fluids and appliances having been sterilized, the dog was prepared for an aseptic incision; the hair was removed with saturated solution of sodium sulphide. The skin was then washed with soap and water, rinsed with alcohol, dried and swabbed with tincture of iodine. An artery and a vein were laid bare and cannulas inserted into each. To prevent necrosis and subsequent sloughing it is desirable to protect the exposed tissues from contact with the anti-coagulent solution.

When working with femoral vessels it is desirable to insert the cannulas about 10 cm. distal to the inguinal ligament. In two instances in which the incision and subsequent ligation were made close to the body gangrene followed. When the incision was made in the neck no untoward results followed ligation of one carotid artery and external jugular vein.

To test the vasomotor irritability intravenous injections of standard doses of "adrenalin" and of nicotine were made. To secure uniform freshness and sterility of the adrenalin it was bought in 1 c.c. ampoules. There was, therefore, little or no variability of the standard due to deterioration in an open bottle. Further to prevent decomposition of the adrenalin, distilled water was used as a diluent. After experimenting with various dilutions and dosages we finally selected as standard 0.66 c.c. and 1.33 c.c. (10 and 20 minims) of 1:100000 solution. It was found that doses below 10 minims gave notably less constant results than those of this size. On the other hand if the vagi are intact quantities larger than 20 to 30 minims are not advisable. With larger doses a reflex inhibition of the heart occurs so that the quantitative relationship between the dosage and its pressor effect is less direct.

The reaction to adrenalin varies greatly according to the speed with which it is injected. To secure uniformity in this respect the following technique was employed: To the venous cannula was connected a reservoir of normal saline solution by means of a rubber tube which was closed by a clip just above the cannula. By means of a hypodermic syringe the epinephrin was quickly

TABLE I

Showing Mean Blood Pressure, Maximum Percentage of Deviation from Average Mean and Maximum Percentage of Deviation from Average in Each Instance. Based upon 46

Dog No.			3	4	7	9	10	11	14	15	16
Weight in kilos			9.5	7.9	?	9.3	13.5	9.4		12.2	10.3
Sex			M	M	F	M	M	M	M	F	F
Blood pressure		R. Femoral	148	120	136	136	108	112	110	120	90
		L. Femoral	150	120	142	132	100	112	118	126	114
		Carotid		122	153		128				
Average			149	120.6	143.6	134	112	112	114	123	102
Maximum deviation			0.7%	1.2%	6.6%	1.5%	14.3%	0%	3.5%	2.4%	11.8%
Adrenalin injection	Dosage [1]		1.33	1.33	2.66	.53	.53	1.33	.53	.80	.80
	Pressor Effect	R. Fem.	8	11	13	18	20	20	32	26	40
		L. Fem.	10	8	10	28	15	24	30	32	40
		Carotid		20	14		16				
Average			9	13	12.3	23	17	22	31	29	40
Maximum deviation			11%	54%	19%	22%	18%	9%	3%	10%	0%
Nicotine injection	Dosage [2]		.30	.66	1.33	.66	.66	1.33	.66		.66
	Pressor Effect	R. Fem.	5	18	18	15	8	14	8		19
		L. Fem.	6		18	14	8	24	14		9
		Carotid		10			8				
Average			5.5	14	18	14.5	8	19	11		14
Maximum deviation			18%	29%	0%	3%	0%	26%	27%		36%

[1] Expressed as c.c. of 1-100,000 solution.

[2] Expressed as c.c. of 1-5,000 solution.

injected into the tube just above the clip. At once this was released and the drug flushed into the vein as rapidly as it could pass through a large-bore cannula under a pressure of two feet of water.

Similarly injections of nicotine were made. The dilution was 1:5000 and the dosage 0.67 c.c. and 1.33 c.c. (10 and 20 minims). After a considerable number of records had been secured these were measured at random and the data pertaining to each animal segregated later. A wire was stretched the length of the record and by inspection the average mean pressure line throughout the experiment determined. The height of this line was then measured. The pressor effect of each drug injection was measured from the highest systolic point at the moment of injection to the

TABLE I

Pressure, Pressor Effect of Constant Doses of "Adrenalin" and Nicotine, Averages of Effects Determinations in 21 Anaesthetized Dogs. All Pressures expressed in Millimetres of Mercury.

22	23	26	27	28	29	31	32	33	34	35	36	Average Deviation
9.8	10	7.8	10.1	9.5	13.6	9.5	7.7	8.2	11.6	11.8	17	
F	M	F	M	M	F	M	M	F	M	M	F	
110	113	100	108	102	136	98	112	126	86	136	128	
108			76	86			110		94	158		
116	114	85			150	98		120			130	
112	113.5	92.5	92	94	143	98	111	123	90	147	129	
3.6%	0.4%	8.1%	17.4%	8.5%	4.9%	0%	0.9%	2.4%	4.4%	7.5%	0.8%	4.8%
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	
28	30	49	23	30	22	38	46	29	24	14	5	
			24	26			23		26	18		
26	26	42			12	33		40			8	
27	28	45.5	23.5	28	17	35.5	34.5	34.5	25	16	6.5	
4%	7%	8%	4%	7%	29%	7%	33%	16%	4%	12%	23%	14%
1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	
9	12	21	30	27	30	26	25		15	14	10	
			26	27			23		17	16		
24	18	35			28	21		32			9	
16.5	15	28	28	27	29	23.5	24		16	15	9.5	
45%	20%	25%	7.1%	0%	3%	11%	4%		7%	7%	11%	15%

highest point attained after it. Following dosages of adrenalin and nicotine of the size employed there results a secondary wave of subnormal pressure. It was thought that these depression waves might have some value as indices of the effects of the injections. The depressor effects, however, were found so variable for given dosages as to be useless as criteria.

A priori one would expect that blood pressure, a product of many variables, would show under the conditions of our experiments a wide variability. The actual determinations show, however, as biologic data go, a very fair degree of constancy. On account of the variability of dosages, weights, and breeds of dogs employed the results in different cases are not comparable with each other. The data are therefore not well adapted to mathe-

matical treatment. The essential point to the experiments, however, is the degree of variability from the average pressure in each case. In Table I is given the two or three pressures noted in each animal, the average pressure and the percentage of the maximum deviation from this average pressure in each dog. The average maximum deviation for the whole series can thereby readily be calculated.

The greatest variability was noted in case of dog 27, in which the higher and lower pressures were 108 mm. and 76 mm. respectively, giving a deviation of 17 per cent from their average value. In half the cases the deviation from the average was within $2\frac{1}{2}$ per cent of that average. The average deviation in the 21 cases was approximately 4.8 per cent from the average pressures.

The reaction to 20 minims of 1–100,000 adrenalin was more nearly constant than to the smaller doses employed. This quantity was finally selected, therefore, as giving the best index of vasomotor irritability. In a few of the earlier determinations, however, only smaller quantities were used. The data were tabulated just as were those previously considered: the pressor effect of each dose, the average in case of each animal, and the maximum percentile deviation from this average are given. Owing to the smaller numerical values the percentile deviations are notably higher than in the preceding series of data. The greatest variation from the average reaction was 54 per cent, in case of dog 4. The average maximum variation of the 21 cases was 14 per cent.

A priori one would expect a relationship between the existing blood pressure and the reaction to the standard dose of adrenalin. Such, however, does not appear in our determinations. Sometimes the reaction was greater with the higher initial pressure and sometimes smaller. In case of dog 27, with initial pressure of 108 mm., the reaction was 23 mm.; but with an initial pressure of 76 mm. the reaction was 24 mm., essentially the same as before.

The effects of the nicotine injections were tabulated just as in the preceding case. The reactions showed a similar degree of constancy. The greatest variability, —45 per cent from the average, was noted in dog 27. The average maximum deviation of the series, 19 cases, was 15 per cent.

In many of the experiments an unexpected sort of constancy

was observed in the reactions to adrenalin and to a less extent in the reactions to nicotine. Following the injections there occurred a series of variable events giving for each animal what might be called a "reaction picture." These "pictures" consisted of various combinations of elevation succeeded by subnormal waves of pressure, of quickened or slowed heart beat, of augmented or depressed pulse pressure and of secondary tension waves. That such a concatenation of features would persist throughout a given series of injections is remarkable. Not only was this true, however, but the same reaction picture was often observed in a succeeding experiment several days later.

A practical difficulty in such experiments is to secure a uniform dosage of the drugs employed. Epinephrin is particularly susceptible to deterioration and the use of the same lot throughout a series of experiments is scarcely feasible. Moreover, the diluted solutions must be made up fresh for each experiment. Considering the high potency of the drug a certain degree of variability of strength of the final dilution is inevitable, even though the laborious plan were followed of starting each time with the dry crystals. Probably no greater error is introduced by using, as we did, a reliable commercial preparation and depending upon the manufacturers' standardization. The results obtained indicate, however, that this source of error is not of major importance.

The experiments as a whole show a fairly satisfactory degree of constancy and indicate that the method used is capable of giving a usable index of vasomotor activity and irritability and a corresponding criterion of the functional condition of the sympathetic nervous system.

Summary and Conclusion

1. Mean arterial blood pressure in the individual anaesthetized dog under laboratory conditions at different times is fairly constant. Forty-six determinations in 21 dogs at intervals of 5 to 10 days showed an average maximum deviation of 4.8 per cent. The greatest individual deviation from the average was 17 per cent.

2. The pressor effect of standard doses of epinephrin injected at different times is proportionately somewhat less constant. The

average maximum deviation from the average was 14 per cent in 21 animals. The greatest individual deviation was 54 per cent.

3. Similar results were secured with nicotine. The average maximum deviation was 15 per cent in 19 animals. The greatest individual deviation was 45 per cent.

4. The reaction consisted of a concatenation of features often giving "reaction pictures" characteristic for each animal.

5. The constancy of blood pressure and of the reactions to epinephrin and nicotine is of a degree to permit their use as criteria of activity and irritability of the sympathetic nervous system.

STUDIES IN FATIGUE

IV. The Relation of Adrenalin to Curare and Fatigue in Normal and Denervated Muscles

By CHARLES M. GRUBER

[*From the Laboratory of Physiology in the Harvard Medical School*]

THAT certain drugs, i.e., atropine and pilocarpine or muscarine, curare and nicotine, curare and salicylate of physostigmin are mutually antagonistic has been shown by different experimenters.

In 1903 Brodie and Dixon,[1] in determining the point of action of adrenalin, used curare for paralyzing the nerve endings in smooth muscle. Very variable results were obtained by its use and they concluded that there was, therefore, a direct antagonistic action between curare and adrenalin, although only to a partial degree. Four years later Panella [2] observed that if curare was injected, either mixed with a small amount of adrenalin or followed by an injection of adrenalin, total paralysis did not result as it did when curare alone was injected.

In this paper I hope to show further that there is this antagonistic action and also that fatigue affects the threshold of a curarized muscle.

The Method

In some cases the animals (cats) were decerebrated, in others they were anaesthetized with urethane (2 gm. per kilo body weight by stomach). In all instances they were tracheotomized. Usually the right tibialis anticus muscle, but in a few cases the left, was used for study.

[1] Brodie and Dixon: Journal of physiology, 1903–04, xxx, p. 497.

[2] Panella: Archives italiennes de biologie, 1907, xlvii, p. 30.

Threshold stimuli were calculated in β units according to the Martin[1] method. The apparatus for this determination was connected to platinum needle electrodes thrust into the muscle. The strength of the primary current for determining the threshold of the normal muscle was .05 ampere and for the curarized and denervated muscle 1.0 ampere.

In every case the arterial pressure was recorded by a mercury manometer connected with the right carotid artery. After adrenalin was injected the blood pressure returned to normal, and after curare was injected the record became horizontal before the threshold was determined.

Through a cannula placed in the left external jugular vein the adrenalin was injected slowly in doses of 0.3 to 2 c.c. of a 1:100,000 solution. Through another cannula, placed in the right external jugular vein, the curare, in a 3 per cent solution, was injected slowly. As soon as natural respiration ceased, artificial respiration was begun and maintained throughout the experiment. For testing the effect of curare the radial nerve or the peroneus communis nerve was stimulated with a strong faradic stimulus.

Experiments were also performed on animals in which a section (2 cm. long) of the left peroneus communis nerve was removed aseptically 7 to 16 days before the experiment.

In experiments in which the muscle was fatigued the stimulating current was a maximal break induction shock obtained from a vulcanite disc interrupter, the rate of stimulation being 120 or 240 times per minute. This rate was kept uniform throughout each experiment. The animals for these experiments were always decerebrated and then fatigued from 10 minutes to one hour.

[1] Martin: Measurement of Induction Shocks, New York, 1912, pp. 71–93. For detailed description of the method employed in this work, see Gruber, this journal, 1913, xxxii, p. 438.

The Normal Threshold Stimulus of Muscle as Affected by Curarè, and the Action of Adrenalin or Fatigue upon the Curare Threshold

Eight experiments were performed in which the average normal threshold for the tibialis anticus muscle varied from 12 to 26 β units or an average of 21 β units. This average is the same as that cited in earlier papers of this series.[1] After intervals varying from 15 minutes to one hour after an intravenous injection of curare, the animals completely immobilized, the threshold stimulus of the eight experiments increased from an average β of 21 to 65.7, an increase of 213 per cent. See Table I.

In the eight experiments performed adrenalin, *in 5 minutes or less*, decreased the average curare threshold of 65.7 β units to 38.9, a recovery of 60 per cent.

Figs. 1 and 2 are curves illustrating the effect of curare and adrenalin. These were plotted from the data of two of the experiments and show the relative heights of the threshold before and after an injection of curare and after an injection of adrenalin. In Fig. 1, points 1 and 2 represent the normal threshold stimuli. After the threshold at 2 was determined 2.5 c.c. of curare was injected intravenously, and 15 minutes later, with the animal immobile, the threshold was again determined (at 3). The injection of curare had increased it from 22.8 β units to 64.9, or 184 per cent. Five minutes after an injection of 2 c.c. of adrenalin (1:100,000) the threshold was decreased (i.e. at 4) from 64.9 to 42.5, a recovery of 53 per cent.

Fig. 2 also shows the relative heights of the threshold before and after an injection of curare and after an injection of adrenalin. In this figure the continuous line is the curve of the normal right tibialis anticus and the broken line that of the denervated left tibialis anticus of the same cat. The peroneus communis nerve was cut seven days previous to the experiment. In Fig. 2 (i.e. at 1 of the continuous line) the normal threshold was 24.3 β units. Eighteen minutes after injecting 3 c.c. of curare, when the animal was paralyzed this threshold was in-

[1] Gruber: this journal, 1913, xxxii, p. 443; *Ibid.*, 1914, p. 335.

TABLE I

THE NORMAL THRESHOLD STIMULUS AS AFFECTED BY CURARE AND THE EFFECT OF ADRENALIN UPON THE CURARE THRESHOLD IN DECEREBRATE CATS. MEASUREMENTS TAKEN BY THE MARTIN METHOD. (I) NORMAL MUSCLE. (II) DENERVATED MUSCLE.

I							II			
Number of c.c. of curare injected 3 per cent	Number of c.c. of adrenalin injected	Normal β	Curare β	Curare β after adrenalin	Increase in per cent	Recovery in per cent	Number of days degeneration	Normal β	Curare β	Curare β after adrenalin
4.5	0.3	25	70	14.1	180	124	7	52.2	50.4	
4	0.4	26.4	72	35.6	173	80	8	93.2	53.8	70.5
3	0.5	24.3	77.6	43.2	219	64	7	50.0	50.8	50.8
3	1	12	41.2	30.7	243	36	8	59.6	53.6	45.8
2.5	2	14.6	47.6	26.1	225	65	14	62.8	60.6	55.3
2.5	1	21.5	124.5	99.6	480	24	8	50.5	50.5	53.0
[1] 3	0.4	21.5	27.8	19.4	29	133				
[1] 1.5	2	22.8	64.8	42.5	184	53				
Average		21.0	65.7	38.9				61.4	53.3	55.1
Increase in per cent of average β = 213						Recovery in per cent of average β = 60				

[1] Urethane anaesthesia.

creased to 77.6 β units (at 2), an increase of 219 per cent. Eighteen minutes later it was again determined and found to be 77.6 β units (at 3). Adrenalin 0.5 c.c. (1:100,000) was then injected intravenously, and six minutes later the threshold (at 4) was found to be 43.2 β units, a recovery of 64 per cent. After 15 minutes' rest the threshold (at 5) was 39.8 β units, and 10 minutes later, after an injection of 5 c.c. of curare, the threshold (at 6) was 64 β units, an increase of 60 per cent.

Since fatigue increases the threshold of a denervated muscle it was interesting to note whether or not it would have an effect upon the threshold of a curarized muscle.[1]

Six experiments were performed. The average β after curare was 60.5. This was increased by fatigue to an average β of 370.6, an increase of 512 per cent. Five minutes or less after an injection of 0.1 to 3.5 c.c. of adrenalin (1:100,000) this threshold was decreased to 176 β units, a recovery of 62 per cent.

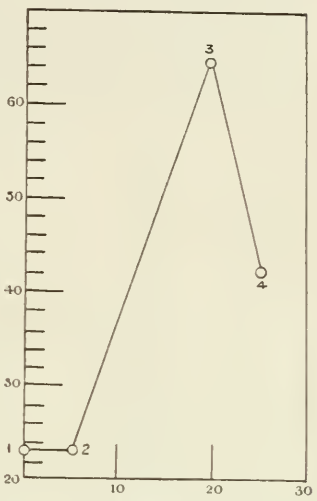

FIGURE 1.—A curve plotted from the data of one experiment. Urethane anaesthesia. The time interval in minutes is represented on the abscissa; the value of the threshold in β units is represented on the ordinate. 1. Normal threshold stimulus 22.8 β units. 2. Threshold after 5 minutes' rest 22.8. 3. The threshold 16 minutes after an injection of 2.5 c.c. of curare, 64.9 β units. 4. The threshold 5 minutes after an injection of 2 c.c. of adrenalin (1:100,000), 42.5 β units.

Does Curare or Adrenalin Affect the Threshold of the Normal Unfatigued Denervated Muscle?

In Table I (II) the average threshold of the denervated muscle, for six experiments, was 61.4 β units. After an injection of curare which completely immobilized the animal the average threshold was 53.3 β units and after an injection of adrenalin the threshold was 55.1 β units. From this table and the broken line in Fig. 2 it is evident that neither curare nor adrenalin affects unfatigued muscles in which the nerve endings are degenerated.

[1] GRUBER: this journal, 1913, xxxii, p. 444; *Ibid.*, 1914, xxxiii, p. 345.

The conditions for testing the denervated muscle were the same as those for the right tibialis anticus, the results of which are shown in the continuous line in the same figure. The slight variations in the curve of the denervated muscle are amply within the limits of error. That there is such marked similarity between the threshold stimuli of the denervated and curarized muscles

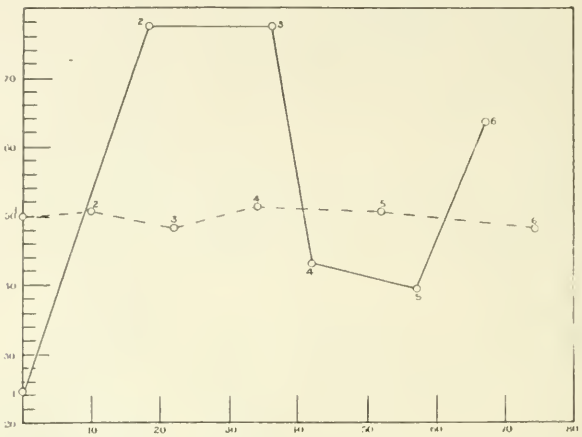

FIGURE 2. — A curve plotted from the data of one experiment performed on a decerebrate cat. The time interval in minutes is represented on the abscissa; the value of the threshold in β units is represented on the ordinate. The continuous line is the curve of the normal right tibialis anticus muscle, the broken line that of the denervated left tibialis anticus (left peroneus communis nerve cut 7 days previous).

The normal muscle: 1. The normal threshold, 24.25 β units. 2. 18 minutes after an injection of 3 c.c. of a 3 per cent solution of curare (animal totally paralyzed) 77.6 β units. 3. After 18 minutes' rest 77.6 β units. 4. The threshold 6 minutes after an injection of 0.5 c.c. of adrenalin (1:100,000), 43.15 β units. 5. After a rest of 15 minutes 39.8 β units. 6. The threshold 10 minutes after an injection of 5 c.c. of a 3 per cent solution of curare 64 β units.

In the denervated muscle: 1. Normal threshold 50 β units. 2. Ten minutes after curare was injected 50.8 β units. 3. 12 minutes later 48.4 β units. 4. 12 minutes later 51.6 β units. 5. 18 minutes after an injection of 0.5 c.c. of adrenalin (1:100.000), 50.8 β units. 6. 22 minutes later or 10 minutes after an injection of 5 c.c. of curare, 48.4 β units.

indicates that seven days are sufficient for degeneration of the severed nerves. The average threshold stimulus of the 14 denervated muscles which were inexcitable on strong faradic stimulation of their cut nerves, was 62.5 β units and that of the curarized animals, in which there was complete immoiblity, was 63.5 β units. See Table II.

TABLE II

The Threshold Stimulus of the Tibialis Anticus Muscle in Decerebrate and Urethanized Cats. Measurements taken by the Martin Method. (I) Peroneus Communis Nerve Degenerated. (II) Curare Paralysis.

Date of the experiment	Number of days nerve cut	Threshold in β units	Date of the experiment	Quantity of curare in c.c.	Threshold in β units
July 7	6	119.2	June 25	2	42.6
[1] July 8	7	83.5	[1] June 26	3	38.5
July 22	7	52.5	[1] June 26	4	80.4
July 23	8	93.2	June 28	1.5	55.2
July 31	7	50.0	June 28	2	73.1
Aug. 1	8	59.6	[1] June 30	2.5	74.8
Aug. 7	14	79.4	[1] June 30	3	27.8
[1] Aug. 18	8	50.5	[1] June 30	1.5	64.9
[1] Aug. 26	7	42.6	July 22	4.5	70
[1] Sept. 3	15	100.1	July 23	4	72
[1] Sept. 5	9	21.5	July 31	3	77.6
[1] Sept. 8	12	36.4	Aug. 1	3	41.2
[1] Sept. 20	14	53.4	Aug. 7	2.5	47.6
[1] Sept. 22	16	32.9	Aug. 18	2.5	124.5
	Average	62.5		Average	63.5

[1] Urethane anaesthesia.

The increase in the normal threshold by curare is probably due to the elimination of the nervous elements. When platinum needle electrodes are thrust into a muscle and the muscle is stimulated electrically, both nerve and muscle tissue are affected. After an injection of curare in sufficient amounts, the irritability of the nerve tissue is eliminated and only muscle tissue is stimulated. The threshold is thus increased. That this is due solely to abolishing nervous influence is shown by the close correspondence

of the average thresholds of curarized muscle and of denervated muscle. See Table II.

Probably curare acts upon a different substance than that upon which fatigue acts. Fatigue increases the threshold of a muscle whether denervated by nerve degeneration, or curare, whereas curare affects only the threshold of a muscle in which the nerve endings are normal.

That adrenalin decreases the threshold of the curarized tibialis anticus has been demonstrated, but the manner in which it exerts this antagonistic action is quite obscure, since the point of action of adrenalin is not definitely known.

Summary

1. Curare increases the threshold of the normal muscle but not the threshold of a muscle in which the nerve endings have degenerated.

2. Adrenalin is an antagonist to curare and decreases, in five minutes or less, the curare threshold, in some cases to normal.

3. Fatigue increases the threshold of a curarized muscle, and adrenalin antagonizes this fatigue.

4. The substance upon which curare acts is probably different from that upon which fatigue acts. It either degenerates upon nerve degeneration, is transformed by muscle atrophy or is inexcitable to electrical stimuli.

VARIATIONS IN THE SENSORY THRESHOLD FOR FARADIC STIMULATION IN NORMAL HUMAN SUBJECTS

III. The Influence of General Fatigue

By E. G. MARTIN, P. R. WITHINGTON, and J. J. PUTNAM, Jr.

[*From the Laboratory of Physiology in the Harvard Medical School*]

In connection with a series of studies being carried on in this laboratory of variations in electro-cutaneous sensibility in normal human beings we have made some observations which seem to show that a state of general fatigue, incident to the daily routine and cumulative from day to day, manifests itself as a progressive general rise in the value of the threshold stimulus. This, in turn, signifies a progressive lowering of sensitiveness and, according to the view of Grabfield and Martin,[1] a diminishing tone of the nervous mechanism as a whole. Our observations indicate further that a pronounced break in the routine may bring about a restoration of sensitiveness to a high point, from which it sinks again as the routine proceeds.

The subjects of our experiments were nine first-year medical students, young adult males in good health. The observations were made in term time. The subjects, therefore, were following a routine which was necessarily regular and similar during six days of each week, made so by the pressure of the school exercises. The routine was interrupted weekly by the Sunday recess, an interval occupied variously by the subjects, but in no case in precisely the manner of the week days.

The sensory thresholds were determined in β units[2] according

[1] Grabfield and Martin: this journal, 1913, xxxi, p. 308.

[2] Martin: The measurement of induction shocks, New York, 1912, p. 76.

to the method described by Grabfield and Martin.[1] Daily readings were made on all the subjects except two between 12.30 P.M. and 1.30 P.M. This period, according to Grabfield and Martin (*loc. cit.*, p. 306), is specially satisfactory for taking readings, since then the sensory threshold is usually at about the position of the average for the day. Two of the subjects were being observed daily at 9.00 A.M. in connection with a special problem. The results obtained from them are included with the others without any attempt at correction for diurnal variation.

In Table I our observations are compiled for convenient reference. To facilitate comparison the data for each day of the week are grouped together. Four vertical columns are devoted to each subject; the first contains the observed thresholds in β units; the second the values of reciprocal $\beta \times 10^4$, used in these studies as indices of irritability[2]; the third the values for irritability reduced to a percentage basis[3]; the fourth the average percentage irritability for each week day. For such studies as this the use of relative or percentage irritabilities, rather than actual irritabilities, is instructive, since the percentage figures not only reveal the extent of departure of each subject from his own average irritability, but also allow direct comparisons between various subjects, whether they happen to have a high or a low general level of sensitiveness.

The data on which we base the conclusion set forth at the beginning of the paper appear in the table in the fourth column under each subject, where the percentage irritabilities for each week day are averaged. These average figures show a well-marked tendency for the irritability to diminish from day to day as the week progresses. There are, as must be expected, departures from this tendency in individual cases, but on the whole, the decline in irritability seems too pronounced to be accidental. Furthermore, the observations were spread over a sufficient number of weeks to avoid the likelihood that irritability changes, interpreted as due to general fatigue, were in reality the result of meteorological or other special conditions.

[1] GRABFIELD and MARTIN: *Loc. cit.*, p. 303.
[2] GRABFIELD and MARTIN: *Loc. cit.*, p. 306.
[3] Ibid, p. 307.

TABLE I

DAILY VARIATIONS IN IRRITABILITY, GROUPED ACCORDING TO DAYS OF THE WEEK

Date	*Subject O.* β	Recip. β × 10^4	% rec. β	Daily av.	*Subject B.* β	Recip. β × 10^4	% rec. β	Daily av.
1913								
Mar. 17,					69.1	145	99	
24					77.6	129	88	
31	76.3	131	116					
Apr. 7	78.8	127	112					
14								
21	88.1	114	101	110	47.1	212	144	107
May 19								
Mar. 18					65	154	105	105
25								
Apr. 1	81.4	123	109	109				
15								
May 13								
Mar. 19					72.3	138	94	
26								
Apr. 2	83.6	120	106	106				
9								
16					65.5	153	104	99
May 14								
Mar. 13								
20					88.2	113	77	
27								
Apr. 3	100.8	99	88					
10	95.7	105	93	90	60.4	166	113	95
May 15								
7					71.1	141	96	
Mar. 14								
21					72.4	138	94	
28								
Apr. 4	98	102	90	90				
11					68.9	145	99	96
18								
May 16								
Mar. 15								
22					77.9	128	87	87
29	94.1	106	94					
Apr. 5	101.2	99	98	91				

TABLE I. (*Continued*)

Date	*Subject L.*				*Subject Wt.*				*Subject H.*			
	β	Recip. $\beta\times10^4$	% rec. β	Daily av.	β	Recip. $\beta\times10^4$	% rec. β	Daily av.	β	Recip. $\beta1\times0^4$	% rec. β	Daily av.
1913												
Mar. 17												
24												
31	93.2	107	86		53.7	188	146		80	125	93	
Apr. 7	55	182	147		90.2	111	86	116	68	147	109	
14												
21	62.2	161	130	121					75.3	133	99	100
May 19												
Mar. 18												
25												
Apr. 1	77.5	129	104	104	78	128	99	99	72.3	138	102	102
15												
May 13												
Mar. 19												
26									69.8	143	106	
Apr. 2	93.3	107	86	86	71.8	139	108	108	66.1	151	112	109
9												
16												
May 14												
Mar. 13												
20												
27												
Apr. 3	92.2	108	87		78.8	127	98	98	64.5	155	115	
10	65.2	153	123	105					66.1	151	112	113
May 15												
7												
Mar. 14												
21												
28					79.6	126	98		80.6	124	92	
Apr. 4	108.3	92	74		92.5	108	84	91	84.7	118	88	90
11	89.7	111	90	82								
18												
May 16												
Mar. 15												
22												
29	86.8	115	93		77.1	130	101		96	104	77	
April 5	99.3	101	81	87	96.6	103	80	90	88.5	113	84	80

TABLE I. (*Continued*)

Date	*Subject Wb.* β	Recip. $\beta \times 10^4$	% rec. β	Daily av.	*Subject Wl.* β	Recip. $\beta \times 10^4$	% rec. β	Daily av.
1913								
Mar. 17					94.9	105	95	
24	87	115	100					
31	65.4	153	133		77.6	129	116	
Apr. 7					111.5	90	81	
14	114	88	76					
21	60.9	164	142	113	79.2	126	113	101
May 19								
Mar. 18					84.8	118	106	
25	100.5	100	87		92.6	108	97	
Apr. 1					73	137	123	109
15	88.3	113	98	92				
May 13								
Mar. 19					64.9	150	135	
26	94.6	106	92		94.8	105	95	
Apr. 2	71.4	140	122	107	95.6	104	94	108
9								
16								
May 14								
Mar. 13					127.5	78	70	
20					109	92	83	
27	100.9	99	86		63.1	158	142	
Apr. 3	98	102	89	87	80.5	124	112	
10					92.1	108	97	101
May 15								
Mar. 7								
14					111.5	90	81	
21					100	100	90	
28	107.5	93	81		95.6	104	94	
Apr. 4	104.1	96	83		93.4	107	96	90
11	64.3	155	135	100				
18								
May 16								
Mar. 15					117.8	85	77	
22								
29	115.7	86	75		93.1	107	96	86
Apr. 5	87.6	114	99	87				
19								
May 17								

TABLE I. (*Continued*)

Date	*Subject G.*				*Subject McG.*			
	β	Recip. $\beta \times 10^4$	% rec. β	Daily av.	β	Recip. $\beta \times 10^4$	% rec. β	Daily av.
1913								
Mar. 17								
24	144	70	92		51	196	105	
31								
Apr. 7								
14	131	76	100	96	51	196	105	
21								
May 19					65	154	83	98
Mar. 18								
25	119	84	110		49	204	110	
Apr. 1	140	71	93					
15	141	71	93	99	47	213	114	
May 13					57	175	94	106
Mar. 19								
26	122	82	108					
Apr. 2	123	81	107					
9								
16	133	75	99	105	45	222	119	
May 14					62	161	87	103
Mar. 13								
20					44	227	122	
27								
Apr. 3	132	76	100	100				
10								
May 15					55	182	98	110
Mar. 7								
14								
21	129	78	103		50	200	107	
28					68	147	79	
Apr. 4								
11								
18	108	93	122	112	42	238	128	
May 16					66	151	81	99
Mar. 15								
22	142	70	92		50	200	107	
29								
Apr. 5	145	69	91		56	179	96	
19	144	69	91	91	69	145	78	
May 17					64	156	84	91

As a means of summarizing our observations the curve shown in Fig. 1 is presented. All the percentage irritabilities for each week day as given in Table I were averaged, and the values thus obtained were plotted against the days of the week with which they correspond. The curve pictures clearly a progressive diminution in sensitiveness which we believe to be characteristic of the human nervous mechanism under such conditions of general fatigue as result from a rather pressing routine.

Although our conclusion is based upon averages, it is supported by direct comparison of the irritabilities of Saturdays and the Mondays following. We have examined 19 such pairs of observations upon the nine subjects of this study and upon two additional subjects. These enable us to compare with the results obtained by the study of averages, the effects on individual cases of the interruption of routine. Of our nineteen observations, twelve showed greater irritability on Monday than on the preceding Saturday; three showed a diminished irritability; and four showed no change. The four cases of unchanged irritability all occurred in three subjects. Of the three cases of lowered irritability two occurred in subjects who at other week ends showed marked increase of sensitiveness. The other case was of a subject upon whom the observations were discontinued before a second week end arrived. The increase of irritability in the twelve cases that showed it, ranged from 7.5 per cent to 82 per cent, averaging 30 per cent; the decrease in the three cases that showed that change, ranged between 7.5 per cent and 15.6 per cent, averaging only 10 per cent.

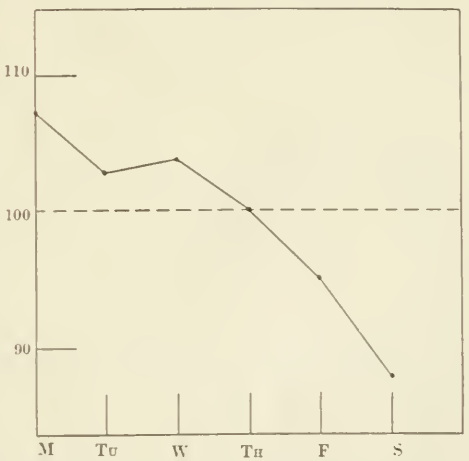

FIGURE 1. Curve of percentage irritabilities showing the general decline in irritability during the week.

In comparison with these observations, and for further assur-

ance that the effects observed are actually associated with the conditions of routine, as we have assumed, we have studied in sixteen cases the irritability on two successive days in the middle of the week. Of these sixteen, eight showed diminished sensitiveness, six increased sensitiveness, and two no change. An interesting fact is that the eight subjects whose irritability change was in the general direction called for by our assumption had an average extent of change of 23.5 per cent, while the six whose irritability change was in the opposite direction had an average extent of change of only 14.9 per cent.

When we consider how many unexplained factors undoubtedly have influence in determining the threshold of sensitiveness from time to time the general relationship we have reported between irritability and routine seems to us undoubtedly significant.

FIGURE 2. Upper curve, the variations in irritability from day to day of a single subject; lower curve, the curve of Figure 1 for general irritability extended to include the same period as does the upper one.

As illustrating the way in which the sensitiveness may follow the routine over a considerable period we reproduce in Fig. 2 the curve of irritability of a single subject, O., from March 29 to April 10, 1913. This subject is a man of very regular habits, and therefore highly favorable for such a study as this one. For comparison our curve of average daily irritability (Fig. 1) is set alongside the other, extended to make it cover the same period.

Summary

Daily observations for several weeks on nine subjects, all following a regular and somewhat pressing routine, show that at the beginning of the week the irritability tends to be high, that from then till the end of the week there is a fairly continuous

decline in irritability, as judged by the sensory threshold, and that following the interruption of the routine by the intervention of Sunday the irritability returns to its original high point.

This is interpreted as a result of general fatigue incident on routine and restoration of nervous tone following a marked interruption therein.

TWO TYPES OF REFLEX FALL OF BLOOD PRESSURE

By E. G. MARTIN and PERCY G. STILES

[*From the Laboratory of Physiology in the Harvard Medical School*]

CHANGES in arterial pressure may be caused by changes in the total volume of the blood, by variations in the output from the left ventricle, or by changes in the peripheral resistance. Alterations of the last-named condition may be due to local influences brought to bear upon the musculature, as in the effect of adrenalin, or to vasomotor mediation. If we limit our attention to the vasomotor factors we recognize that a dilation with lowering of resistance to the blood-stream may be occasioned by a lowering of the tonic activity of the vasoconstrictor centre or, at another time, by the intervention of vasodilator impulses. Presumably the widest possible opening of the vascular channels will be secured when vasoconstrictor tone is abolished and the dilators are universally stimulated.

With regard to the afferent side of the mechanism by which the calibre of the small blood-vessels is changed we have had a very clear conception of the several possibilities ever since their presentation by Hunt[1] in 1895. A fall of pressure, reflexly brought about, may be the result of reduced tonic activity on the part of the vasoconstrictor centre; in this case the afferent fibres concerned are said to have a depressor effect. Such a fall may be the expression of dilators coming into play; in this case it is appropriate to call the afferent fibres excito-dilators. Excito-dilator reflexes need not be assumed to involve a localized vasodilator centre, no such anatomical feature being known. Evidently, a given sensory nerve may contain both depressor and excito-dilator fibres, or the same fibres may make central connections such as to mediate both reactions.

[1] Hunt: Journal of physiology, 1895, xviii, p. 381.

The best known example of a nerve which can be relied upon to produce a reduction of blood-pressure when its central end is stimulated is found in the group of afferent fibres commonly called the Depressor.[1] This appears in the rabbit and some other animals as a strand accompanying the vagus. It is assumed to be represented in other cases by a set of fibres within the vagus trunk. These fibres have been said to originate in the wall of the aorta and to be stimulated by the distension of that vessel near its root. Thus a rise of aortic pressure tends to secure a compensatory adjustment and to be promptly abated.

The Depressor nerve, or the depressor fibres, have been believed to effect a lowering of arterial tension partly through reflex inhibition of the heart but more extensively in the animals usually chosen for experiment by suppressing the tonic action of the vasoconstrictor centre. If the reaction were confined to this mode of operation the name Depressor (by which depressor par excellence seems to be implied) would be fully justified. Sherrington[2] has with great ingenuity brought this central action into the class of reciprocal innervation. He points out that the circular muscle elements in the blood-vessels are the true antagonists of the cardiac elements and that it is to be expected that an increased action of the heart will be attended by an inhibition of the vascular musculature, just as the contraction of a flexor is accompanied by the inhibition of the corresponding extensor.

But the actual working of the Depressor is not limited to the inhibition of existing vasoconstrictor tone. There is an enlisting of vasodilators in the response and a certain share of the loss of pressure is to be referred to this action. Bayliss[3] was led to believe this even before the work of Hunt mentioned above had been published. In 1893 he expressed his opinion that one can discover traces of the dilator effect in the presence of the true depressor phenomenon. Much later[4] he studied the submaxil-

[1] Ludwig and Cyon: Berichte d. k. Gesellschaft, math. phys. Classe., Leipzig, 1866, p. 307.

[2] Sherrington: The integrative action of the nervous system, Scribners, New York, 1906, p. 99.

[3] Bayliss: Journal of physiology, 1893, xiv, p. 314.

[4] Bayliss: Journal of physiology, 1908, xxxvii, p. 264. Also Fofanow and Tschallusow: Archiv für die gesammte Physiologie, 1913, cli, p. 543.

lary gland in this connection with clear and interesting results. The constrictor supply of this gland may be cut off by severing the cervical sympathetic. After this has been done, stimulation of the depressor still accelerates the flow from the vein of the gland, a fact which must be accounted for on the assumption of a positive excitation of the dilator fibres in the chorda tympani. The converse experiment — cutting the chorda and obtaining a real depressor reaction from the gland — is also successful.

Bayliss noted that the type of pressure reduction which he referred to the dilators was moderate in degree and not lasting. The profound lowering seen when the stimulation of the Depressor is adequate may be continued for a long time with little or no exhibition of a tendency toward a recovery of normal pressure. Bayliss suggested that the transient action of the dilator mechanism might be held to indicate central fatigue and that fatigue is something which we expect to see in connection with an excitatory process. An inhibitory process, on the other hand, might not entail any fatigue because of its negative character; it certainly should not on the Gaskell conception of anabolic processes in the cells involved. At any rate, the main effect of depressor stimulation is one which may be maintained without flagging for as long as 17 minutes.

The present paper is to report the results of an attempt to bring the Martin method for the physiological calibration of induction shocks[1] to bear upon the analysis of the depressor reaction. These experiments have been upon cats. In this animal a separate Depressor is said to be frequently found. Our experience does not confirm such a statement although the number of animals observed has been small. Out of some fifteen cats we have seen in two cases only, a slender nerve alongside the vagus which would have been taken for a Depressor on anatomical grounds. In both these cases stimulation of the nerve in question failed to give the typical depressor reaction and this was found to be obtainable by stimulating the main trunk of the vagus. We have once seen the complete failure of the usual effect when

[1] Martin: The measurement of induction shocks, Wiley, New York, 1912.

the central end of the vagus was excited and in this instance the power was found to reside in the nerve of the opposite side.

Our procedure has been to anaesthetize, sometimes with ether and at other times with urethane. Tracheotomy has then been performed and both the vagi have been cut. Stimulation has been by means of a Sherrington electrode connected with a calibrated induction coil. Break shocks have been given by means of a mechanical, motor-driven interrupter at rates between 8 and 15 per second. The blood pressure has been taken in most cases from the femoral artery. It is better to use this vessel than the carotid because if the latter is chosen there is danger of wetting the nerve with the fluid used to retard coagulation, in this research a strong solution of sodium carbonate.

FIGURE 1. — Showing the slight increase of effect upon the blood pressure following stimuli rated as 22, 35, and 60Z (1, 2, 3 in the record) and the profound effect of the stimulus of 127Z (4), this being above the Depressor threshold.

The strength of the stimulation employed was deduced from the position of the secondary coil and the amperage of the primary current. Our figures are therefore in the Z-units of Martin [1] and are not corrected for variations of external resistance. Under this system one reading can be fairly compared with another so long as the electrode remains in one position; the readings cease to be accurately comparable otherwise, and yet their range from one experiment to another continues to have a general value.

[1] MARTIN: *loc. cit.*, p. 73.

It is to be remembered that we are dealing with a series of nerves which vary in size and physical condition only within rather narrow limits.

A series of typical results may now be described. If we begin with stimuli too weak to affect the blood pressure and proceed to apply stronger and stronger ones we shall presently obtain a response which is recorded as a transient lowering of pressure. This is the reaction interpreted by Bayliss as a reflex through the dilators. There is usually a partial recovery within the period of 30 seconds during which we ordinarily continue to stimulate. With gradually increasing intensity of stimulation through a considerable range this "mild" reaction is repeated with but little augmentation. Often there is an "all or none" character about it which is quite suggestive.

Since we are making use of a mixed nerve and not a pure Depressor such as can be found in the rabbit, there is, of course, the possibility of reflexes from the skeletal muscles, particularly those concerned in the breathing movements. But as a matter of fact these disturbances have very seldom become noticeable. Nearing a certain level we begin to see hints of the change from the mild to the profound depression of the blood pressure and when the threshold is cleared the effect quickly becomes maximal. The fall of pressure is now a matter of 25 per cent or more and there is no rebound within the stimulation period of 30 seconds. It is to be noted that the cutting of the other vagus has subtracted from the possible depression that fraction which could have been produced in the normal animal through reflex inhibition of the heart. A further increase of stimulation above that giving the typical depressor effect adds but little to its magnitude.

Here we have very distinctly the appearance of two thresholds. The lower of these is measured by the strength of stimulation necessary to secure the "mild" or supposed excito-dilator response. How wide is the interval between this and the higher one, assumed to be the threshold for inhibition of the vasoconstrictor centre, will be made plain by the following table. The attempt to assign precise values to these thresholds does not lead to satisfactory results. The full depressor reaction does not appear all at once when the record first shows a departure

from the excito-dilator curve; there is a relatively narrow range within which the influence of changing stimulation is a graded one. One reason why the thresholds cannot be very sharply defined is found in the shifting due to modified conditions accompanying repeated trials.

Our chief results may be conveniently grouped as below:

54 trials in which the average stimulus was 10Z gave an average drop of 6 per cent.

59 trials in which the average stimulus was 70Z gave an average drop of 8 per cent.

28 trials in which the average stimulus was 175Z gave an average drop of 8.5 per cent.

54 trials in which the average stimulus was 250Z gave an average drop of 27 per cent.

The entrance of the second, or profound, effect is evident. If we were to add a fifth group comprising the stimuli which were manifestly supra-maximal, the average pressure reduction would still be in the vicinity of 27 per cent.

Attention may again be called to the approximately "all or none" character of the mild or assumed vasodilator reaction. It appears in the tabulation above that an increase of strength of stimulus from 10Z to 175Z, that is, a 17-fold multiplication, makes the sagging of the blood-pressure only slightly more pronounced. We could cite one series after another, chosen from the records averaged in our summary, in which the weak stimuli were fully as efficient as those many times stronger. This is so striking as to make it desirable to have a comparison between these vagal reflexes and those obtainable from other nerves. We are able to introduce the desired figures for the sciatic, the saphenous, and for certain branches of the brachial plexus.

The application of stimuli to the central end of the sciatic can be depended upon to cause a moderate lowering of pressure so long as the stimuli are below a certain intensity. Using this nerve we passed on one occasion, by many gradations, from a stimulation strength of 11Z to 45Z and obtained with the weakest stimulus a drop of 8.5 per cent and with the strongest a fall of 8.3 per cent. The intermediate values were all between 5.4 and

8.9 per cent. In another experiment the change of pressure remained between 10 and 11 per cent while the stimulation was shifted from 35 to 86Z. Again, the effect varied only between 10.6 and 11.1 while the stimulation was changed in strength from 41 to 145Z.

When the saphenous nerve has been under observation it has been the general experience that very strong stimuli have given no greater reduction of pressure than could be secured by the employment of others much weaker. This is a small nerve and it is doubtless more subject to injury than are the others we have used. For this reason somewhat less dependence is to be placed upon the quantitative findings based upon its investigation. But when due allowances are made the results still appear to be significant. For example, in a series of six trials in which the weakest stimulus was 3.25Z and the strongest 360Z the effect of the first was a fall of 8.1 and of the last 8.2 per cent. The intermediate stimulations gave magnitudes varying between 2.9 and 9.2 per cent, a wide fluctuation but entirely unrelated to the stimulation gradient. In another experiment a descending series of five trials gave the following results: for 60Z a drop of 9.6 per cent, for 11Z a drop of 9.1 per cent. The three intermediate figures for the pressure change are 10.7, 8.9, and 10.9 per cent.

The nerves of the fore-limb are probably more reliable for our purpose. From these we have obtained such results as the following: in one instance a stimulus rated as 5Z produced a fall of 9.1 per cent, 11Z gave 9.2 per cent, 22Z gave 7.0 per cent, and 360Z gave 11.6 per cent. In another protocol we find that 5Z gave a fall of 5.3 per cent, 11Z gave 4.0 per cent, and 60Z 4.1 per cent.

Reviewing our data we are not disposed to claim that the excito-dilator reaction has an all or none character in precisely the same sense that the heart muscle has that property. When we use this expression with reference to the heart we mean that the least stimulus capable of evoking any response causes a full-sized contraction. In this case we believe rather that the excito-dilator reaction is a graded one from its threshold through a limited range of stimulation, but that a maximal effect is

soon attained and that the stronger stimuli are then supramaximal.

Martin and Lacey[1] have recently reported that with afferent nerves other than the vagus a depressor reaction is exchanged for a pressor effect when the stimulation is carried above a threshold of reversal which they have found to be near 280Z. The figures we have cited for the vagus show that our second type of reaction is superimposed upon the first when the strength of the shocks applied is of a roughly similar order. (The difference between the position of the secondary coil for a stimulus of 250Z and that necessary to give 300Z is but a few millimetres.) The obvious suggestion is that the threshold for the inhibition of the vasoconstrictor centre is the same as for its excitation. In the solitary case in which we could not elicit the depressor property from the vagus we found that this nerve like others produced pressor reactions when strongly stimulated. The reversal occurred in the vicinity of a stimulation strength of 400Z.

Our attention was called by Doctor Cannon to the possibility that the mild type of depression might be due to a reflexly induced discharge of adrenalin of the order of magnitude which Cannon and Lyman[2] found to cause vasodilation. It seemed to us that the response was rather too prompt to be accounted for in this way, but we made a precautionary experiment in which the adrenal bodies were excluded from the circulation. We found that we could still obtain the familiar reduction of 5 to 10 per cent on applying weak stimuli to the vagus.

[1] Martin and Lacey: this journal, 1914, xxxiii, p. 212.
[2] Cannon and Lyman: this journal, 1913, xxxi, p. 376.

THE INFLUENCE OF FOOD, POSTURE, AND OTHER FACTORS ON THE ALVEOLAR CARBON DIOXIDE TENSION IN MAN

By HAROLD L. HIGGINS

[*From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.*]

Introduction

THE tension of CO_2 in the alveolar air, representing the CO_2 tension in the arterial blood, was shown by Haldane and Priestley [1] to be the normal regulator of respiration. It was further shown by these authors and later more extensively by Fitzgerald and Haldane [2] that the alveolar CO_2 tension of the same individual was practically always the same, although individuals differed markedly from each other as to their normal CO_2 tension. But under abnormal or unusual conditions the CO_2 tension has been found to vary from the normal. Especially is this the case when there is an acidosis, or increase of acid in the blood; in such conditions there is a lower alveolar CO_2 tension. This has been noted in diabetics [3 4] and earlier in cases of oxygen want [5 6] and especially at high altitudes.[7] This has led to the presentation of the theory,[8] recently experimentally confirmed by Hasselbalch [9] that it is the

[1] Haldane and Priestley: Journal of physiology, 1905, xxxii, p. 225.

[2] Fitzgerald and Haldane: Journal of physiology, 1905, xxxii, p. 486.

[3] Beddard, Pembrey, and Spriggs: Journal of physiology, 1908, xxxvii, p. xxxix.

[4] Porges, Leimdorfer and Markovici: Zeitschrift für klinische Medizin, 1911, lxxiii, p. 389.

[5] Boycott and Haldane: Journal of physiology, 1908, xxxvii, p. 355.

[6] Haldane and Poulton: Journal of physiology, 1908, xxxvii, p. 390.

[7] Ward: Journal of physiology, 1908, xxxvii, p. 378.

[8] Winterstein: Archiv für die gesammte Physiologie, 1911, cxxxviii, p. 167.

[9] Hasselbalch: Biochemische Zeitschrift, 1912, xlvi, p. 403.

H-ion concentration of the arterial blood which is the main factor in the regulation of breathing. In this paper I shall deal with variations in the alveolar CO_2 tension in man found after the ingestion of food and with the subject in different postures, which are difficult to explain by changes in the quantity of acids in the blood other than H_2CO_3, and which point to another factor in the regulation of respiration.

Method

The Haldane[1] method of determining the alveolar air was used in the cases quoted in this paper. In this method one collects and analyzes a sample of the last part of an expiration, which is alveolar air. To get information as to the average composition of the air in the alveoli, two samples are taken, one at the end of inspiration, when the CO_2 content is naturally lowest, and the other at the end of expiration, when it is highest. The technique of the method requires quite normal breathing for a minute or more; then at the end of a normal inspiration, the subject expires rapidly and fairly deeply through a long tube of about three-quarters inch (19 mm.) diameter; at the end of this expiration the subject seals the tube with his tongue (or a valve is used to close the end through which he breathed); a sample of the air in the tube near the mouthpiece is then taken and later analyzed. Similarly another sample is taken when the rapid deep expiration is made at the end of a normal expiration. The chief criticism of and difficulty in the Haldane method has been that the subject, being conscious of his breathing, is unable to breathe naturally before the taking of a sample; a deeper breath just before the sample is taken leads to a too low result. In experimenting on new subjects for the first time one often notices this tendency to breathe deeply, which when two determinations are made, one a few minutes after the other, generally leads to widely varying results. But with nearly every subject after the first day satisfactory samples are obtained, as practically everybody can learn to breathe naturally, even while conscious of his respiration. As

[1] Haldane and Priestley: *loc. cit.*

a check that the breathing has been normal before taking the samples, most of the figures given here are the average of two or more determinations; the duplicates usually agree very closely (within one part in forty). In some of the earlier experiments of this series but one sample of the alveolar air was taken, that after a normal inspiration; the differences found in the alveolar air when one sample was taken were all verified by later experiments where the average of the two samples was taken; in fact the relation between the inspired and expired samples at rest was found to be very constant.

The subjects in these experiments were all young men from twenty to thirty years of age, most of them being medical students and none of them being engaged in heavy muscular work.

To show the variations in the normal alveolar CO_2 tension in subject over a period of several months, the following figures are given for two subjects; all the results are with the subject sitting and in the breakfastless (nüchtern) condition, and each figure represents the average for one day.

H. — 38.5, 38.5, 37.6, 39.9, 37.7, 39.0, 39.5, 40.0, 37.9, 39.4, 38.7, 37.9, 38.9, 39.9, 38.9, 38.4, 37.4, 36.6, Av. 38.6 mm. (Hg) CO_2 tension (i.e., $\pm$ about 2 mm.).

M. — 41.0, 43.5, 43.4, 42.6, 42.4, 42.8, 41.8, 41.7, Av. 42.4 mm. (Hg) CO_2 tension ($\pm$ about 1 mm.).

Thus as noted by Haldane and Priestly, there is a distinct, but different average for each of the two subjects. There are small but distinct differences on different days with the same subject.

To show about how much variation may be expected on the same day with a subject, the following figures are given, which are the alveolar CO_2 tensions on successive hours (9 A.M. to 4 P.M.) when no food was taken.

H. — 39.5, 37.6, 36.9, 38.7, 39.6, 38.9, 38.2, 38.8, Av. 38.5 mm. (Hg) CO_2 tension ($\pm$ 1.6 mm.).

No marked diurnal variations were noticed.

Effect of Taking Food upon the Alveolar CO_2 Tension with the Subjects in a Sitting Position

Porges, Leimdorfer, and Markovici mention that the CO_2 tension of the blood [1] was raised above the nüchtern value after the subject had taken breakfast. The higher CO_2 tension of the blood after food is ascribed by these authors to the flow of gastric juice in the stomach. The reasoning is that with the withdrawal of HCl from the blood, the latter becomes more alkaline; therefore, a higher tension of CO_2 is required to keep the H-ion concentration of the blood at a point sufficiently high to stimulate the respiratory centre. But in this connection one must remember that although acid is taken from the blood to form gastric juice, alkali is also taken to form pancreatic juice (in amounts more than enough to neutralize the gastric juice). While it is true that the gastric juice begins to flow before the pancreatic juice, it is not very much over 15 minutes under any circumstances, and even less with liquid nourishment. But owing to the earlier flow of the gastric juice and the fact that for a time this loss of acid will be felt only by the blood and not by the body fluids as a whole, one must concede that this may be the cause of the raising of alveolar air. But on the other hand, if the blood became much more alkaline by reason of the flow of gastric juice, one would expect that the urine would become more alkaline immediately after eating; but that this is not invariably the case, has been shown by Hasselbalch.[2]

Table I gives the results of experiments made where the

[1] *Loc. cit.* Their method, which represents the CO_2 tension of the venous blood rather than that of the arterial blood, gives a value about 20% higher than the alveolar CO_2 tension; but the changes noted in their paper with a resting subject are probably coincident with similar changes in the alveolar CO_2 tension.

[2] *Loc. cit.* Tables II and III in Hasselbalch's article show almost invariably a higher acidity (H-ion concentration) in the urine for the period (No. 2) immediately after a meal than for the preceding breakfastless period (No. 1). Results (unpublished) by Dr. Smillie of the Harvard Medical School show similar tendencies for the period following food. Determinations in several of the experiments reported here show no marked difference between the breakfastless and food periods.

TABLE I

EFFECT OF FIRST MEAL OF DAY UPON THE ALVEOLAR CO_2 TENSION, SUBJECT SITTING

Subject	Date 1913	Before food (mm. Hg)	After food (mm. Hg) ¼ hr.	½ hr.	¾ hr.	1 hr.	1½ hr.	2 hr.
Br.	Mar. 17	38.8	—	45.0	—	44.5	—	44.1
Bz.	Feb. 5*	39.4	42.6	41.5	—	41.8	40.2	—
R.	Apr. 11	37.0	—	—	—	—	—	39.4
U.	5	34.8	—	—	—	37.6	—	—
H.	Feb. 8	37.6	40.0	39.3	38.5	40.3	39.7	40.6 [1]
	28	36.2	38.5	—	—	41.0	40.6	—
	Mar. 6	39.5	—	—	—	—	—	—
	7	37.8	—	—	—	41.1	—	— [2]
	8	40.0	—	—	—	41.4	—	—
	20	39.4	—	41.3	—	—	42.0	— [3]
	21*	38.7	—	—	—	41.6	—	—
	Apr. 3	38.9	—	—	—	—	42.0	—
	May 1	38.4	—	—	—	41.3	—	—
	Dec. 20	36.6	—	40.2	—	—	40.7	—
M.	Feb. 10	41.1	42.6	42.6	—	43.0	44.2	44.0 [4]
	19*	43.5	—	45.0	—	45.6	45.9	—
	Mar. 6*	42.8	—	44.5	—	44.4	—	44.4 [5]
	19	41.8	—	43.4	—	42.6	—	43.3 [6]

[1] 2½ hr. 39.8 [2] 3 hr. 39.9.

[3] A second meal was taken 2 hr. after the first and the alveolar CO_2 continued 1 hr. 40.3; 2 hr. 41.2.

[4] 2½ hr. 45.2. A second meal was taken 3 hr. after the first and the alveolar CO_2 continued ½ hr. 44.4; 1 hr. 43.8; 2 hr. 43.3; 3 hr. 44.4.

[5] 3 hr. 45.8; 4 hr. 44.1; 5 hr. 43.9; 6 hr. 43.3.

[6] 3 hr. 42.8; 4 hr. 44.1.

NOTE. — On days starred, coffee was taken.

alveolar air was taken before and at varying intervals after the taking of food. The rise of the alveolar CO_2 tension after food is very evident in practically every case. In Table II a similar rise is noted after a non-carbohydrate meal in the case of normal individuals where an acidosis had been produced. The presence of carbohydrate in the diet is thus obviously not essential to this rise, although generally considered as the important factor in reducing an acidosis. The rise is still manifest after one and one-half hours in every case but one.[1]

The effect of a second meal on the alveolar air, while the effect of the first meal was still evident, is shown in two cases; no further rise is evident, showing the effect of food is not cumulative.

TABLE II

EFFECT OF THE FIRST MEAL OF THE DAY UPON THE ALVEOLAR CO_2 TENSION, WHERE THE MEAL CONTAINS NO CARBOHYDRATES

Subject and date (1912)	(mm. Hg)								
	H			Ha.	K.	S.		C.	
	Nov. 6	7	8	Dec. 22	Dec. 28	Dec. 29	30	Dec. 29	30
Before food	35.8	31.0	30.1	39.2	28.4	37.6	37.1	39.9	38.4
After food [1]	38.8	33.4	31.9	39.1	30.3	40.3	40.9	40.7	40.6
No. of days immediately preceding on which subject has had no carbohydrate	0	1	2	0	1	1	2	1	2

[1] 1–1½ hr.

Sugar. When 100 g. of cane sugar was taken, the rise in the alveolar CO_2 tension was again found, but it lasted for a little less than an hour.

[1] Compare HASSELBALCH: *loc. cit.*

TABLE III

EFFECT ON THE ALVEOLAR CO_2 TENSION OF TAKING 100 GRAMS OF CANE SUGAR SOLUTION

Subject	Date (1913)	(mm. Hg)					
		Before taking sugar	After taking sugar				
			¼ hr.	½ hr.	1 hr.	1½ hr.	2 hr.
H.	Feb. 18	37.4	39.0	39.2	37.8	37.8	36.6
	Mar. 18	37.9	39.9	38.6	37.8	37.7	38.9 [1]

[1] At the close of this experiment food was taken, and the alveolar CO_2 tension rose to 41.3 mm.

Beefsteak. The effect of eating a small beefsteak was tried. The cooking was done in the room with the subject, and alveolar air determined during that period. But no rise in the alveolar CO_2 tension was noticed which might be ascribed to psychic flow of the gastric juice, although after eating, the CO_2 tension rose as usual.

EFFECT OF 80 GRAMS OF BEEFSTEAK ON THE ALVEOLAR CO_2 TENSION

Before 37.4 mm. During cooking 37.6 mm. After eating (½ hr.) 38.8 mm.; (1½ hours) 40.1 mm.; (2 hours) 39.0 mm.

Agar-agar. Agar-agar offers the opportunity of producing digestive movements without absorption, with perhaps also some flow of digestive juices. The alveolar CO_2 tension may have become a little higher, but by no means so markedly as with absorbable food.

Summarizing in brief the experiments thus far presented, one may say that there is a rise in the alveolar CO_2 tension after taking food of any sort lasting in general as long as there is active absorption (for example ¾ hr. with sugar and 4–6 hr. with less quickly absorbed food). While the rise is probably proportional to the bulk of absorbable food taken, if the total amount is small, yet it does not rise above a certain maximum. The rise is not due to the existence of an acidosis in the breakfastless state, as

TABLE IV

EFFECT ON THE ALVEOLAR CO_2 TENSION OF TAKING 10 GRAMS AGAR-AGAR

Subject	Date (1913)	(mm. Hg)				
		Before taking	After			
			½ hr.	1 hr.	1½ hr.	2 hr.
H.	Feb. 8	39.0	39.0	40.3	39.8	—
	15	38.5	39.1	37.6	38.6	39.8 [1]
M.	12	43.5	44.1	43.6	—	43.0

[1] 2½ hr. 39.2; 3 hr. 38.3. Food taken 3½ hr. after the agar-agar raised the CO_2 tension to 41.6 mm.

immediately after the absorption of food ceases, the breakfastless value is again obtained.

EFFECT OF POSTURE ON THE ALVEOLAR CO_2 TENSION

Experiments, where the alveolar carbon dioxide tension of the same subject was determined in different positions, are summarized in Table V. The postures chosen were standing, sitting, lying on the side, on the back, and in the Trendelenburg position.[1] A comparison was also made of the alveolar carbon dioxide tension of a subject sitting in a reclining and in an erect position.

The experiments seem to show clearly that the alveolar CO_2 tension is lowest when the subject is standing, higher when sitting, and still higher when lying. One can find no decisive difference between the values obtained lying on the back and on the side; but in the Trendelenburg position, the alveolar CO_2 tension is decidedly lower than in the other lying positions and approximates that of sitting. Sitting erect leads to a lower alveolar CO_2 tension than sitting in a reclining position.

The general conclusion to be drawn is that the more relaxed the position, the higher the alveolar CO_2 tension. In one case,

[1] I am indebted to Prof. Y. HENDERSON for suggesting that this position be included.

TABLE V

ALVEOLAR CO_2 TENSIONS IN DIFFERENT POSITIONS (BREAKFASTLESS)

Subject	Date (1913)	(mm. Hg.)					
		Standing	Sitting	Lying Trendelenburg	Lying on back	Lying right side	Lying left side
F.	Dec. 18	37.4	—	—	40.6	—	—
	19	37.2	—	—	38.3	—	—
Br.	Mar. 17	37.3	38.8	—	41.8	—	—
R.	Apr. 11	37.2	37.0	—	40.1	42.5	—
U.	5	36.5	34.8	36.6	35.7	—	—
H.	Feb. 18	—	37.6 [1]	—	39.5	—	—
	26	36.8	39.9	—	41.6	—	—
	28	34.4	36.2	—	39.7	—	—
	Mar. 6	36.5	39.5	—	—	—	—
	8	36.6	40.0	—	—	—	—
	31	—	39.4	38.8	40.8	—	40.8
	Apr. 3	—	38.9	40.2	41.3	42.2	42.3
	May 1	35.2	38.4	40.2	40.5	41.0	—
	Dec. 13	33.5	—	—	39.9	—	—
Average		**35.5**	**38.8**	**39.7**	**40.6**	**41.6**	**41.6**
M.	Feb. 19	39.1	42.8 [2]	—	43.8	—	—
	21	38.8	42.8	—	44.3	—	—
	Mar. 19	40.3	41.8	—	42.6	—	—
	Apr. 2	40.7	41.7	41.9	42.8	42.0	42.2
Average		**39.7**	**42.3**	**41.9**	**43.4**	**42.0**	**42.2**

[1] Sitting erect 37.0; sitting reclining 38.2.
[2] Sitting erect 41.8; sitting reclining 43.8.

U., these differences were not noticed, but with the other subjects the results seem consistently to point to this general conclusion. The order in which the different positions were taken made no difference in the result; the alveolar CO_2 tension for any of the positions assumed its level for the position almost immediately on taking it; alveolar CO_2 tensions determined after three to five minutes were essentially the same as those determined after the subject had been in the position for a longer time. That the changes in the alveolar air due to different postures are not the result of nervous impulses carried from the diaphragm to the respiratory centre, seems to be indicated by the failure to find the highest value in the Trendelenburg position, where there is the largest pressure on the diaphragm.

The results of the experiments on the alveolar CO_2 tension in other positions than sitting when food was taken are given in Table VI. One finds the rise in the CO_2 tension standing after taking food to be fully as large as when the subject is sitting. However, when the subject is lying, the rise is not so large on the average; although in some of the cases it is fully as large as sitting, yet in others there is little or no rise at all.

Effect of other Factors on the Alveolar CO_2 Tension

Coffee. Experiments were made on the alveolar CO_2 tension as the result of taking coffee. About 350 c.c. of black coffee were taken in each experiment.

TABLE VII

Effect of Coffee on the Alveolar CO_2 Tension

Subject	Date	Before taking	After taking				
			$\frac{1}{4}$ hr.	$\frac{3}{4}$ hr.	1 hr.	2 hr.	$3\frac{1}{2}$ hr.
H.	Mar. 21	38.7	39.3	—	37.3	36.0	38.7
	Oct. 23	37.4	36.8	35.6	34.8	36.0	38.3

A fall in the CO_2 tension is noticed in both experiments; but the lowering effect of coffee, as shown in these experiments, was not great enough to overcome the rise in the alveolar CO_2 tension due to the taking of food in previously cited experiments in Table II.

TABLE VI

EFFECT OF FOOD ON THE ALVEOLAR CO_2 TENSION WITH THE SUBJECT IN OTHER POSITIONS THAN SITTING

Subject	Date (1913)	Standing				Lying on back			
		Before food	After food			Before food	After food		
			¼–1 hr.	1–2 hr.	2–3 hr.		¼–1 hr.	1–2 hr.	2–3 hr.
Br.	Mar. 17	37.3	40.2	40.1	40.6	41.8	42.7	43.8	43.1
R.	Apr. 11	37.2	—	39.5	—	40.1	—	42.5	—
H.	Feb. 28	—	—	—	—	39.7	41.6	40.7	40.9
	Mar. 6	36.5	—	37.5	38.5	—	—	—	—
	8	36.6	39.1	37.4	—	—	—	—	—
	Apr. 3	—	—	—	—	41.3 [1]	—	—	42.6 [2]
	May 1	35.2	—	39.3	—	40.5 [3]	—	43.3	— [4]
M.	Feb. 19	—	—	—	—	43.8	—	43.3	—
	Mar. 1	—	—	—	—	41.8	42.4	40.7	43.0
	19	40.3	41.3	40.5	40.7	42.6	44.5	46.3	45.8
F.	Dec. 18	37.2	39.9	41.2	—	40.6	42.3	43.9	—
	19	37.4	—	40.4	—	38.3	41.9	41.7	—

[1] Lying right side 42.2; left side 42.3; Trendelenburg 40.2.
[2] Lying right side 43.9; left side 44.5; Trendelenburg 42.9.
[3] Lying right side 41.0; Trendelenburg 40.2.
[4] Lying right side 42.8; Trendelenburg 41.9.

Aromatic Spirits of Ammonia. — The effect on the alveolar CO_2 tension of smelling aromatic spirits of ammonia for a half minute was tried. In the case of K., who a few moments previously had recovered consciousness after fainting, the smelling of the aromatic spirits of ammonia caused the alveolar CO_2 tension to drop from 42.4 to 38.9 mm.; this drop was coincident with the return of color to the face. In the case of H. (who was not faint) the drop was not so great, being from 39.6 to 38.6 mm.

Discussion

While it is perhaps not yet possible to lay down any hard and fast rule as explaining the results obtained, there are many coincidences in connection with these experiments that it is well to note. It seems highly probable that the rise in the alveolar air following the intake of food and that following the taking of a more relaxed position have much in common. It is well known that drowsiness is very often noticed after a heavy meal and also that it is more prone to appear in a reclined or relaxed position. Thus, one may conclude that drowsiness is more likely to be evident with a high alveolar CO_2 tension. One finds vasodilation in the skin or splanchnics, the former in a relaxed position and the latter after food; thus it will appear that the higher alveolar air is also coincident with vaso-dilation; where there is vasodilation from both food and position, as after food when in a lying position, there is not noticed in some cases marked cumulative effect to raise the alveolar CO_2 tension by reason of both factors. The fact that the rise in the alveolar CO_2 tension lasts only so long as there is active digestion and absorption of food, and the fact that coffee, a vasoconstrictor, lowers the alveolar CO_2 tension, furnish additional evidence to point to the fact that vasodilation and a high alveolar CO_2 tension and vasoconstriction and a low alveolar CO_2 tension run parallel. The anatomical position of the two centres — the respiratory and the vasomotor — confirm the possibility that the same impulse might affect both at the same time.

Hasselbalch [1] reports that the H-ion concentration of the blood, other things being equal, increases with the concentration of the blood corpuscles; unless there is a diminution of the blood corpuscles in the blood entering the respiratory centre in a relaxed position or after food, it seems that some other factor beside the H-ion concentration must be exerting an influence upon the respiratory centre.

[1] *Loc. cit.*

Conclusions

1. The alveolar CO_2 tension rises after the intake of food and remains high so long as the food is in active digestion.

2. The alveolar CO_2 tension is higher when one is in a relaxed position than when one is in an erect position. Thus the alveolar CO_2 tension is markedly higher standing than sitting, and higher sitting than lying.

3. The taking of coffee, without food, caused a fall in the alveolar CO_2 tension.

4. These variations, especially those from changing position, do not appear to be due to changes in the H-ion concentration of the blood (independent of the H_2CO_3); but apparently some other agent is affecting the respiratory centre to cause these changes.

5. A high alveolar air is coincident with vasodilation, and a low alveolar CO_2 tension with vasoconstriction.

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THE ANTERIOR LOBE OF THE PITUITARY BODY IN ITS RELATIONSHIP TO THE EARLY GROWTH PERIOD OF BIRDS

By ROSALIND WULZEN

[*From the Rudolph Spreckels Physiological Laboratory of the University of California*]

PATHOLOGICAL conditions in the pituitary body have been so frequently connected with abnormal growth that the former are generally conceded to bear a causal relation to the latter. Nevertheless attempts to modify growth by increasing the normal amount of pituitary secretion present in the body have yielded various and somewhat ill-defined results.

Caselli[1] noted no effect on growth after long-continued injections of whole pituitary glycerine extracts but found that ingestion retarded growth in some instances. Foderà and Pittau[2] observed that emaciation resulted from injection of pituitary extracts. Sandri[3] fed large quantities of ox pituitary to young mice for a period of two months and found that there was a notable arrest of growth. He also injected guinea pigs with a pituitary emulsion and found again a diminution in the rate of growth. He fails to state that he used control animals. Crowe, Cushing, and Homans,[4] using boiled suspensions of powdered pituitary from either dog, pig, or ox, found that repeated injections of the entire body caused rapid loss in weight both in puppies and in adult dogs. Pure anterior lobe preparations had no such

[1] Caselli: Revista sperimentale di freniatria. Reggio-Emilia, 1900 xxvi, pp. 176, 486.

[2] Foderà and Pittau: Gazzetta di medicina e chirurgia, 1909, viii, p. 149.

[3] Sandri: Archives italiennes de biologie, 1909, li, p. 337.

[4] Crowe, Cushing and Homans: Bulletin of the Johns Hopkins Hospital, 1910, xxi, p. 127.

effect, a normal puppy being given daily injections for three months without visible result. Also, according to Cushing,[1] Goetsch and Cushing found that one puppy fed daily with three grain doses of powdered extract of whole pituitary was less in height at the end of the experiment than the control.

Cerletti[2] worked upon guinea pigs and rabbits, using whole pituitaries of young sheep from which he prepared a glycerine emulsion. He injected an amount about equal to three fourths of a whole pituitary into the peritoneal cavity every five or six days. He also used dogs, giving them a more frequent dosage. All of his experiments covered considerable periods, the longest being 144 days. His results were uniform throughout. During the period of the experiment the animals receiving injection of pituitary extract fell constantly below the controls in weight. Moreover in the case of the dogs, measurement of the hind legs showed that those of the control were increasingly greater in length throughout the experiment than those of the dogs receiving pituitary injection. Measurement of the tibia in two experiments with rabbits showed that the diaphyses of the control animals were longer, but that the frontal diameter of the epiphyses of the animals receiving pituitary injection was equal to or greater than that of the controls.

Schäfer[3] fed young rats on a constant small amount of dried anterior lobe added to a measured diet of bread and milk. Fresh ox pituitaries were kept for a few days in chloroform, were then separated into anterior and posterior lobes, finely divided and dried. The controls were fed upon similarly prepared ovary or testicle. A group of four rats was fed in this way for three months. For the first eight days the pituitary fed rats fell below the controls in weight, but at the close of the experiment their total weight was almost twice that of the controls. In a second series of experiments similarly conducted, Schäfer[4] arranged the animals in three groups, the first containing four young females, the second three young males, the third three half grown males,

[1] Cushing: The pituitary body and its disorders, 1912.

[2] Cerletti: Archives italiennes de biologie, 1907, xlvii, p. 123.

[3] Schäfer: Proceedings of the Royal Society, 1909, lxxxi, B, p. 442.

[4] Schäfer: Quarterly journal of experimental physiology, 1912, v, p. 203.

each group being duplicated by a control group. The experiment continued for three months. At first ¾ gm. of material was administered. This amount was doubled after two months and still further increased toward the end of the experiment. At the conclusion the ovary fed animals in the first two groups were a little heavier than the pituitary fed animals. In the third group the pituitary animals about equaled the ovary fed animals in weight. The conclusion is drawn from these experiments that the addition of small amounts of ovarian or pituitary tissue to the diet of rats has little or no effect upon growth.

Aldrich[1] performed two series of experiments, the first upon dogs, the second upon rats. He added to the bread and milk diet of each about 50 mg. per day of fresh, dessicated, defatted anterior lobe of ox pituitary. He used seven dogs divided into two groups, one group being fed upon similarly prepared ovary as control. The average weights show that the controls had a natural tendency to increase in weight more rapidly than the pituitary animals, but for certain reasons Aldrich concludes that there is neither stimulation nor retardation in growth, though in individual cases the anterior lobe may stimulate. In the second series, Aldrich[2] used ten young rats divided into two groups, male and female in each. The control group, fed as in the case of dogs, held an increasingly greater weight than the pituitary group during the three months of the experiment. Ingestion of the anterior lobe thus impedes growth. A similar series performed with posterior lobe gave no such inhibition. Aldrich mentions that J. L. Miller has conducted experiments on young white rats in much the same way and has obtained negative results as regards weight and skeletal change.

The preponderance of the above evidence indicates that the pituitary body either injected or ingested is able to cause a diminution in rate of growth in young animals. That the falling off in weight is due to something more than emaciation has been shown by those investigators who have through measurement of the long bones found a decrease in their length. I have been

[1] Aldrich: This journal, 1912, xxx, p. 352.
[2] Aldrich: This journal, 1913, xxxi, p. 94.

able to confirm in this series of experiments both the falling off in weight of the animal and in length of bone following ingestion of anterior lobe of the pituitary body.

Experimental

In order that dependable averages might be secured through the use of a number of individuals of the same age, the domestic fowl was selected for the investigation. The work was started September 25 with two groups each containing eighteen White Leghorn chicks either two or nine days old at that time. Feeding with pituitary material began October 3 and continued until January. The chicks were kept within a small enclosure in the laboratory and given artificial heat until they reached 250 gm. in weight when they were allowed the range of a sunny room. They were fed at first upon finely cracked grains with a little boiled rice, then upon coarsely cracked grains, entire wheat, grit, and a mash composed of bran, shorts, bone meal, cornmeal and charcoal. They were also given green feed. Five days in the week each chick was fed a weighed amount of unmodified anterior lobe of ox pituitary or in the control group an equal amount of fresh liver. The ox pituitaries were obtained from the Oakland Meat and Packing Company through the great courtesy of the Superintendent. The glands were eaten by the chicks within twenty-four hours after being removed from the cattle at the slaughter house. The amount of pituitary material given varies throughout the experiment, the attempt being to keep it roughly equal to one hundredth of the average body weight of the pituitary chicks. A few individuals died early in the experiment. During December probably on account of the confinement and adverse weather conditions, the chickens contracted roup. On this account it was thought best to give figures for two months only and to gather the data from those individuals which up to December appeared normal. If all had been used the result would have been to reduce the averages of the pituitary fed individuals still further, as the very smallest individuals in the pituitary group were eliminated in this way, while those eliminated from the controls were of fairly large size. It should also be observed that

the results obtained during the first two months were continued without change during the third month. The extension of the curves in the direction in which they are going would indicate in a general way the growth occurring in this last interval.

The results here presented are based upon data gathered from twenty-five individuals divided into four groups: (1) 7 pituitary fed females, (2) 10 liver fed females, (3) 4 pituitary fed

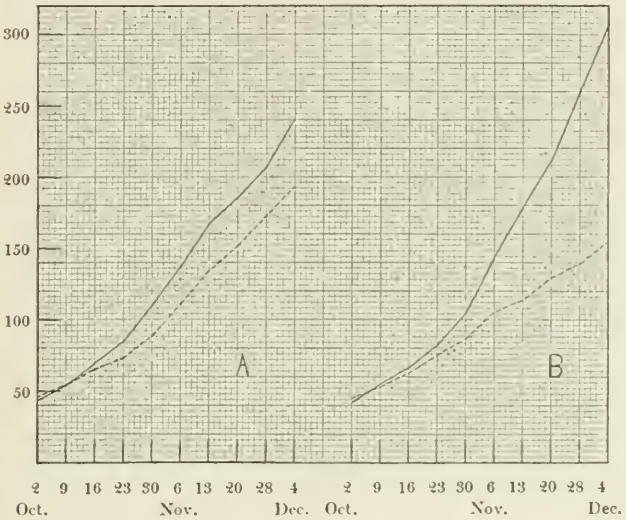

FIGURE 1. Derived from Table I. Abscissae: successive dates. Ordinates: weight in grams. Broken lines: data from pituitary fed chickens. Solid lines: data from controls. The curves represent the average weight in successive weeks of (A) 7 pituitary fed females and 10 control females, (B) 4 pituitary fed males and 4 control males.

males, (4) 4 liver fed males. Measurements were taken at weekly intervals. Table I, represented graphically by the accompanying curves (Figs. 1 and 2), gives for each group (1) the average weight in grams, (2) the average length of wing in millimeters, (3) the average length of foot in millimeters. The length of wing was measured by placing the joint between radius and metacarpus against an upright and measuring on a horizontal meter stick to the tip of the feathers. The foot was measured in a similar manner from the joint between tibio-tarsus and tarso-metatarsus to the end of the central toe.

All the measurements show a distinct inhibition of growth in

the pituitary fed chickens both male and female. It is noticeable that the males are more affected than the females. At the conclusion of the experiment, while the average measurements of the male controls were greater than those of the female controls, the average measurements of the pituitary fed males were less than those of the pituitary fed females. The measurements of all

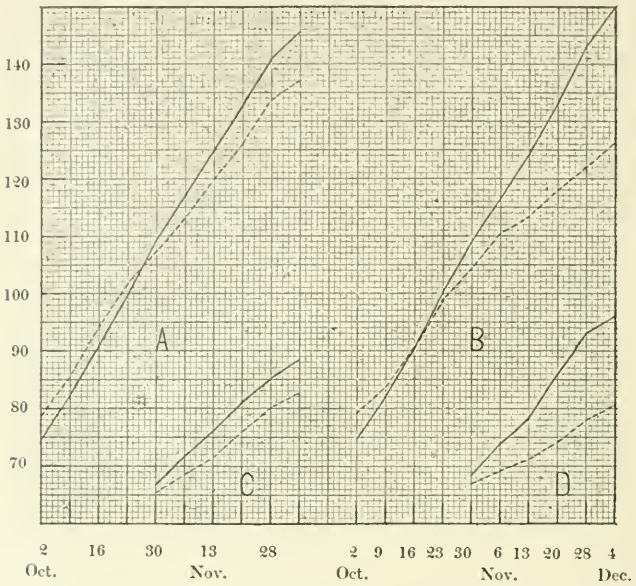

FIGURE 2. Derived from Table I. Abscissae: successive dates. Ordinates: length in millimeters. Broken lines: data from pituitary fed chickens. Solid lines: data from controls. The curves represent the average length in successive weeks of (A) the wings of 7 pituitary fed females and 10 control females, (B) the wings of 4 pituitary fed males and 4 control males, (C) the feet of 7 pituitary fed females and 10 control females, (D) the feet of 4 pituitary fed males and 4 control males.

the individuals included in the totals for December 4 present this fact in another way (Table II). It will be observed that whereas the two groups of females overlap one another in weight and length of bone, the male groups show no overlapping in any measurement. This difference could hardly be entirely due to the smaller number of males.

The inhibition of growth indicated in the tables was easily apparent on inspecting the living animals. Those fed upon

TABLE I

Date	Grams Pituitary or Liver fed	Female						Male					
		Pituitary fed Average of 7			Control Average of 10			Pituitary fed Average of 4			Control Average of 4		
		Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.
Oct. 2	.3	45.4	78.7		43.5	74.8		45.0	79.2		41.5	74.7	
" 9	.4	54.1	85.8		53.9	82.4		53.0	83.7		55.5	82.2	
" 16	.6	65.4	94.2		69.6	90.9		62.7	90.7		65.7	90.5	
" 23	.8	73.2	101.7		84.5	99.8		75.2	99.0		81.5	100.5	
" 30	.9	88.7	107.4	65.4	109.9	109.3	66.9	86.0	104.5	67.0	104.0	109.0	68.5
Nov. 6	1.1	111.7	113.2	68.5	136.9	117.2	71.7	105.2	110.5	69.2	144.0	116.5	74.0
" 13	1.3	133.8	120.0	71.4	167.3	124.8	76.1	113.2	113.5	71.2	178.5	124.2	78.2
" 20	1.3	151.8	126.2	76.0	185.7	132.9	81.0	129.0	118.0	74.2	211.5	133.2	86.0
" 28	1.4	173.2	133.7	80.1	207.3	140.9	85.1	139.5	122.2	78.0	261.0	143.2	93.2
Dec. 4	1.5	192.8	137.1	82.7	240.3	145.5	88.5	154.0	126.2	80.5	308.0	150.5	96.2

pituitary material were noticeably smaller, this difference being especially prominent in the males. After three months, the three smallest males, all pituitary fed, showed no wattles and

FIGURE 3. Photograph taken November 29. The posterior chicken is a control male. The anterior one is a pituitary fed female. Note how the control exceeds in length of foot, wing and tail and in height. The female could have been exchanged for a male in the same group without altering the appearance of the picture.

FIGURE 4. Photograph taken about January 15. The larger chicken is a control male, the smaller a pituitary fed male. Besides differences in size of all parts of the body, note that the pituitary fed chicken has no wattles and a very small comb as compared with the well developed comb and wattles of the control.

their combs were only slightly larger than those of the females, whereas the combs and wattles of the three remaining control males were large and well developed. (Figs. 3 and 4.)

These results are reinforced by a previous feeding experiment in which three White Leghorn fowls were raised to adult size. One, the control, was a male. The others, fed as above on pituitary material, were male and female. This male originally exceeded the control in size, but in the course of a few days it dropped below and remained smaller than the control during the period of growth corresponding to that covered by the tables given above. The female was always the smallest of the three.

Involution of the thymus. After two months' feeding, the smallest of the pituitary fed chickens died. Autopsy showed that there was practically no thymus tissue left. For comparison the smallest of the controls was killed. It was found to have a considerable amount of thymus tissue stretching through the neck in close contact with both jugular veins. This led to an

TABLE II

Female Pituitary fed			Female Control			Male Pituitary fed			Male Control		
Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.	Wt. gm.	Wing mm.	Foot mm.
129	128	73				124	134	85			
134	125	72				156	118	80			
			162	133	79	157	123	76			
			185	137	84	179	130	81			
194	135	87							206	142	95
196	140	85							322	148	96
			202	134	83				347	160	95
			218	139	88				357	152	99
			222	139	86						
231	142	85									
			232	150	81						
238	150	87									
			254	155	92						
			279	157	96						
			320	163	99						
			329	148	97						

examination of the thymus in the living chickens. Long areas of skin free from feathers extend along the dorsal aspect of the chicken's neck, and by parting the feathers to lay these bare and also stretching and moving the skin it is possible to see both jugular veins quite plainly with their attendant masses of thymus tissue. Although some of the thymus which lies close to the thorax may be overlooked, a good idea of the amount of thymus tissue may be gained in this way. By means of the examination, which was made in the early part of December, it was found

possible to arrange the chickens in a somewhat accurate series from the one possessing the smallest to the one possessing the largest thymus. This agreed in general with the order of arrangement by weight including both males and females. Table III shows the distribution of the pituitary and the control chickens according to the size of the thymus. On weighing the total

TABLE III

Group I Thymus small	Group II Thymus somewhat larger	Group III Thymus medium	Group IV Thymus large
7 pituitary 3 males 4 females	3 pituitary 1 male 2 females	1 pituitary female	
1 control female	3 controls 1 male 2 females	4 controls all females	6 controls 3 males 3 females

thymus tissue in representatives of Groups I, II, and IV, it was found that the specimen thymus from Group I (body wt. 148 gm., thymus wt. .1038 gm.) was .07% of the body weight. In group II, it was .14% of the body weight (body wt. 229 gm., thymus wt. .321 gm.). In Group IV it was .31% of the body weight (body wt. 299 gm., thymus wt. .953 gm.). Thus there is more than four times as much thymus tissue per gram of body weight in the representative of Group IV as in the representative of Group I. It will be noticed that Group IV is made up entirely of control animals whereas all the small thymuses except one belong to pituitary fed animals. Here again the males will be seen to be more widely separated than the females, all the male pituitary chickens having small thymuses whereas all the male controls had very large thymuses except the one in Group II which soon after became sick.

Although I can find in the literature no observations concerning the involution of the thymus under pituitary feeding, the converse condition has been observed. Aschner[1] notes that the

[1] Aschner: Archiv für die gesammte Physiologie, 1912, cxlvi, p. 1.

thymus persists abnormally in young dogs from which the pituitary has been removed, which is in accordance with the retention of certain infantile characteristics. The same observation has been made by Cushing,[1] who further adds that clinical experience

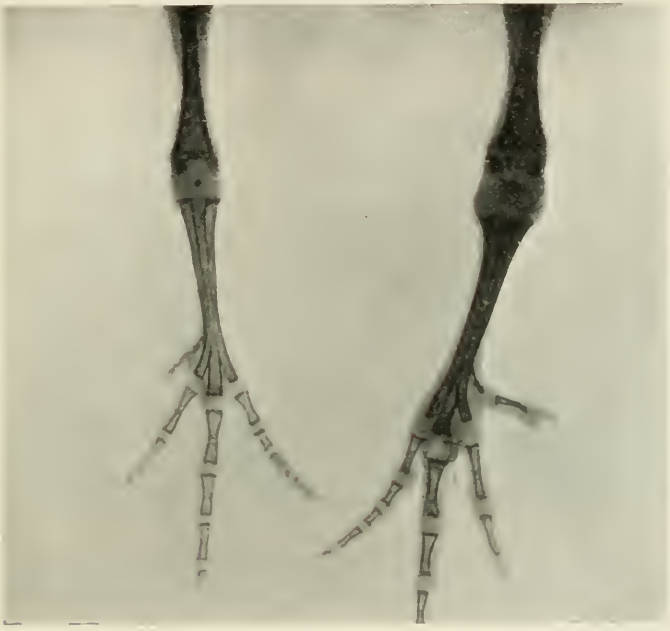

FIGURE 5. Radiogram taken December 5. The larger leg is that of a control female, the smaller that of a pituitary fed male. At the time each was the closest representative of the average in its own group. Note the greater length of bone in the foot and the larger and more plainly defined centers of ossification at the tarsal joint in the control.

would lead him to believe that in cases of hypophyseal insufficiency there is apt to be a persistent and enlarged thymus where the process dates from a preadolescent period.

It is possible that the diminution in growth which accompanies injection and ingestion of pituitary tissue may be caused indirectly by disturbances in nutrition. But it is also possible that this inhibition may be the result of a primary effect of pituitary secretion upon the thymus. In fact the changes brought about through

[1] CUSHING: *Loc. cit.*

its action call to mind the changes found by Basch[1] to follow extirpation of the thymus in young dogs. In the operated animals the bones were slighter and smaller than in the controls while the epiphyseal lines were frequently broadened and irregular. There is a larger proportion of cartilaginous tissue at the epiphyseal line than in the normal animals. The cartilaginous covering of the epiphyses is thicker and more voluminous and the

FIGURES 6 and 7. Radiograms taken from two of the chickens raised to adult size in a previous experiment as mentioned in the text. Figure 6 is the tarsal joint of a pituitary fed female about five months old. Figure 7 is the tarsal joint of a control male of the same age. Compare especially the distal ends of the tibio-tarsus. That of the pituitary fed hen shows considerably more cartilage and less bone than that of the control, suggesting the changes in bony growth found by Basch to follow extirpation of the thymus.

ossified bony portion is smaller. The long bones take longer to develop and this causes a marked retardation in growth of the whole body. These words might be used in large part as a description of the differences existing between the bones of pituitary fed chickens and controls as shown by the accompanying radiograms. (Figs. 5, 6, and 7.) Whether or not the above idea is fruitful will require further experimentation to decide, and it is my purpose to continue the study.

[1] BASCH: Jahrbücher für Kinderheilkunde, 1906, lxiv.

Conclusions

1. The growth of young fowl is retarded by the addition to the diet of fresh, unmodified anterior lobe of ox pituitary. This is shown both in body weight and in length of the long bones.

2. Involution of the thymus accompanies this retardation and may bear a causal relation to it.

3. These effects are more marked in the males than in the females.

The writer extends grateful thanks to Professor Maxwell for his constant and kindly suggestions and help.

THE RÔLE OF NASCENT OXYGEN IN REGULATING THE ACTIVITIES OF ENZYMES IN ANIMALS AND PLANTS

By W. E. and E. L. BURGE

[*From the Physiological Laboratory of the University of Illinois*]

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IT may be shown in several ways that nascent oxygen destroys all the ordinary enzymes.

We have found that ptyalin, amylopsin, malt diastase, rennin, pepsin and trypsin are destroyed by the passage of the direct electric current and that the rate of this destruction is directly proportional to the amount of current used. Since the time of Faraday it has been known that the passage of the direct electric current decomposes water and that the amounts of oxygen and hydrogen liberated are directly proportional to the amount of current passed. We have no evidence that nascent hydrogen decreases the activity of enzymes, but we have abundant evidence that nascent oxygen decreases their activity and that this decrease is more or less proportional to the amount of oxygen used. Hence it seems reasonable to conclude that the cause of the destruction of enzymes by the passage of the direct electric current is oxidation.[1]

The above named enzymes are also destroyed by bubbling oxygen gas or air through their solutions, but the rate of this destruction is very slow. However, if a piece of platinum mesh, previously covered with platinum black, be introduced into the solution, thereby supplying nascent oxygen, the rate of the destruction of the enzyme is greatly increased.[1]

We have been able to show in still another way that nascent oxygen destroys the activity of enzymes. If hydrogen peroxide be added to the enzyme solution and a piece of platinum mesh,

[1] Burge: Résumés des Communications, IXième Congrès international des Physiologistes, Groningue. 1913.

previously covered with platinum black, be introduced the oxygen liberated from the hydrogen peroxide by the platinum black oxidizes the enzyme. This method was used in the experiments to be described in this paper.

The solutions were made by dissolving one gram of a commercial preparation of the enzyme in 15 cubic centimeters of distilled water. Such solutions possessed optimum activity. Two cubic centimeters of the enzyme solution were used in each experiment. This amount was diluted to 7 cubic centimeters using varying amounts of hydrogen peroxide and a diluting solution. The diluting solution consisted of some of this solution of hydrogen peroxide which had been decomposed completely by means of platinum black. The strengths of the diastatic enzymes were determined by the amounts of reducing sugar produced by the addition of 2 cubic centimeters of the solution to 10 cubic centimeters of a 0.2% starch paste. The digestion was carried on in the case of amylopsin and ptyalin for three minutes at 38° C. In the case of malt diastase and taka diastase the amounts of starch paste and of the enzyme solution were the same as for amylopsin and ptyalin, but the digestion was carried on for thirty minutes at

TABLE I

Ezyme	N	A	B	C	D	E
	2 c.c. enzyme 5 c.c. diluting solution 0 c.c. H_2O_2	2 c.c. enzyme 4 c.c. diluting solution 1 c.c. H_2O_2	2 c.c. enzyme 3 c.c. diluting solution 2 c.c. H_2O_2	2 c.c. enzyme 2 c.c. diluting solution 3 c.c. H_2O_2	2 c.c. enzyme 1 c.c. diluting solution 4 c.c. H_2O_2	2 c.c. enzyme 0 c.c. diluting solution 5 c.c. H_2O_2
Amylopsin	9.8 mgs.	6.8 mgs.	5.0 mgs.	2.6 mgs.	0.0 mgs.	0.0 mgs.
Ptyalin	7.5 "	7.3 "	7.5 "	7.2 "	2.4 "	0.0 "
Malt Diastase	9.4 "	5.4 "	4.2 "	2.8 "	0.0 "	0.0 "
Taka Diastase	8.9 "	8.4 "	8.6 "	8.9 "	8.7 "	8.8 "
Pepsin	1.4 mm.	1.2 mm.	1.2 mm.	1.0 mm.	0.8 mm.	0.2 mm.
Rennin	30″	50″	60″	75″	540″	8 hrs.

60° C. Pavy's method was used for the estimation of the sugar. Mett's tubes were used in determining the strength of the pepsin solutions. The activity of the rennin was determined by adding 1 cubic centimeter of the solution to 5 cubic centimeters of fresh cow's milk at 38° C.

The details of these experiments may be seen in the accompanying table.

The solutions indicated in column N were made by adding 5 cubic centimeters of the diluting solution to 2 cubic centimeters of the enzyme solution of optimum activity. The solutions in column A were made by adding 4 cubic centimeters of the diluting solution and 1 cubic centimeter of hydrogen peroxide to 2 cubic centimeters of the concentrated enzyme solution. Solutions in column B were the same as those in column A except that 3 cubic centimeters of the diluting solution and 2 cubic centimeters of hydrogen peroxide were used. The solutions indicated in columns C, D and E were similar to those in A and B except for the increasing amounts of hydrogen peroxide and the decreasing amounts of diluting solution as shown in the table.

The experiments were made as follows: A piece of platinum mesh, previously covered with platinum black by the passage of the direct electric current, was thoroughly washed and introduced into a solution of amylopsin made by adding 5 cubic centimeters of the diluting solution to 2 cubic centimeters of the concentrated amylopsin solution (column N). This was allowed to stand for thirty minutes. It will be noticed that no oxygen was given off to this solution. Two cubic centimeters of this solution were added to 10 cubic centimeters of a .2% starch paste and permitted to stand at 38° C. for three minutes. At the end of this time the digestion was brought to a close by bringing the solution to boiling in thirty seconds. It may be seen in column N for amylopsin that 9.8 milligrams of reducing sugar were present as determined by Pavy's method. The same piece of platinum mesh was introduced into another solution of amylopsin made by adding 4 cubic centimeters of the diluting solution and 1 cubic centimeter of hydrogen peroxide to 2 cubic centimeters of the concentrated solution of amylopsin. This solution was permitted to stand until the 1 cubic centimeter of hydrogen peroxide was completely de-

composed by the platinum mesh. The diastatic power of this solution was then determined as before and in column A it may be seen that this was represented by 6.8 milligrams of reducing sugar. In column B for amylopsin may be seen the result of a similar experiment except that 3 cubic centimeters of hydrogen peroxide were used. The diastatic power of the amylopsin was here represented by 5.0 milligrams of reducing sugar. The experiments in column C, D and E for amylopsin were made in a similar way with the solutions indicated in the table. In each of these solutions was placed a piece of platinum mesh and each was allowed to stand until all the hydrogen peroxide was decomposed. In column C for amylopsin the diastatic power of the solution is represented by 2.6 milligrams of reducing sugar, and in column D the diastatic power is reduced to O. In column C the solution was exposed to the oxygen liberated from the decomposition of 3 cubic centimeters of hydrogen peroxide. In column D the solution was exposed to the oxygen liberated from 4 cubic centimeters of hydrogen peroxide. This amount was sufficient to oxidize completely the amylopsin present.

It may be seen that the normal diastatic power of the amylopsin used in this experiment is represented by 9.8 milligrams of reducing sugar, and that when a similar amount of amylopsin was exposed to the action of the oxygen liberated from 1 cubic centimeter of hydrogen peroxide its diastatic power was reduced to 6.8 milligrams of reducing sugar, i.e., the amount of oxygen that can be liberated from 1 cubic centimeter of hydrogen peroxide reduced the diastatic power of the amylopsin by 3.0 milligrams of reducing sugar. It will be observed for amylopsin in column B that the oxygen liberated from 2 cubic centimeters of hydrogen peroxide reduced the diastatic power by 4.8 milligrams of reducing sugar, or 2.4 milligrams of sugar per cubic centimeter of hydrogen peroxide. In column C the oxygen from 3 cubic centimeters of hydrogen peroxide reduced the diastatic power by 7.2 milligrams of reducing sugar, or 2.5 milligrams per cubic centimeter of hydrogen peroxide. In column D the oxygen liberated from 4 cubic centimeters of hydrogen peroxide completely destroyed the dastatic power of the amylopsin.

Similar experiments to those on amylposin were carried out on

ptyalin, malt diastase, taka diastase, pepsin and rennin. It may be seen for ptyalin in column N that when 2 cubic centimeters of the concentrated solution of ptyalin were diluted to 7 cubic centimeters by adding 5 cubic centimeters of the diluting solution the diastatic power is represented by 7.5 miligrams of reducing sugar; that when a similar amount of a solution of ptyalin was exposed to the action of the amount of oxygen liberated from 1 cubic centimeter of hydrogen peroxide the diastatic power is represented by 7.3 milligrams of reducing sugar and that when it was exposed to the amount of oxygen liberated from 5 cubic centimeters of hydrogen peroxide by platinum black its diastatic power was reduced to 0. It will be noticed that the rate of the destruction of ptyalin was not proportional to the amount of hydrogen peroxide added and hence not to the amount of oxygen liberated. It may be seen also that a similar destructive action is produced on malt diastase by the oxygen liberated from hydrogen peroxide. Its diastatic power was completely destroyed when its solution was exposed to the amount of oxygen liberated from 4 cubic centimeters of hydrogen peroxide. The solutions of taka diastase were not affected by the amounts of oxygen used in these experiments. However, the activity of taka diastase was destroyed by exposure to the amount of oxygen liberated from 12 cubic centimeters of hydrogen peroxide. The activity of both pepsin and rennin was greatly reduced but was not completely destroyed by the amount of oxygen obtained from 5 cubic centimeters of hydrogen peroxide. Both of these enzymes were destroyed by the oxygen liberated from 7 cubic centimeters of this hydrogen peroxide. The rates of the destruction of these two enzymes run parallel. This may be accounted for by the fact that the solutions of pepsin and rennin were both made from the same commercial preparation of pepsin. We do not think that the result is any proof for the identity of the two substances because if a commercial preparation be chosen which has strong rennetic property and weak peptic property it is possible to destroy the peptic property without apparently affecting the rennetic.

In addition to the enzymes enumerated in the table we have found that oxygen liberated from hydrogen peroxide by platinum black destroys the diastase extracted from Elodea canadensis

gigantea, trypsin, emulsin, invertase, bromalin, papain and autolytic enzymes.

Brown and Morris[1] have shown that the amount of diastase in the leaves of foliage plants increases during the night and decreases during the day. Using Elodea canadensis gigantea, a green water plant, we have been able to confirm their observations. We also found that the amount of destruction of the diastase was more or less proportional to the length of time of exposure of the plant to light. The fact that diastatic enzymes are destroyed by nascent oxygen would seem to offer an explanation of this observation that the diastase in plants decreases during the exposure of the plant to light. The assumption would be that the oxygen liberated during exposure to light oxidizes the diastase formed, hence a decrease in diastatic activity during the day. As soon as the plant is removed to darkness oxygen ceases to be given off and during this period the diastatic activity increases.

The oxidative action of the tissues has been demonstrated by various observers[2] employing a variety of reactions by which colored oxidation products are formed within the tissues. These observers[3] have shown that the capacity of the tissues to form these colored products bears a direct relation to their capability of freeing oxygen from hydrogen peroxide, of bluing tincture of guaiac and of oxidizing salicylic aldehyde and benzyl alcohol to their respective acids. They found that there are definite and constant differences in the oxidative properties of the different tissues. This property was most marked in the spleen, liver and kidney and least marked in muscular and nervous tissue.

Using the above methods Lillie[4] examined the oxidative properties of the different regions of the alimentary canal. When such sections were placed in a solution of alpha benzol and di-amidobenzene the parts of the cross section where oxidation takes places most rapidly were stained violet by the formation of indophenol dyes. He found that when cross sections of the stomach wall were placed in such solutions the mucous membrane assumed a

[1] Brown and Morris: Journal of the Chemical Society, 1893, 63, p. 604.
[2] Medwedew: Archiv für die gesammte Physiologie, 1896, 65, p. 249.
[3] Salkowski (mit Jamagiwa): Virchow's Archiv, 1897, 147, p. 1.
[4] Lillie: This journal, 1902, 7, p. 413.

deep violet coloration which was particularly intense at the inner ends of the cells. The muscular layers took on a diffuse and relatively slight coloration in accordance with the generally observed feeble oxidative properties of muscle. The connective tissue portions of the sub-mucosa remained almost colorless. Cross sections of regions of the intestine showed a similar distribution of the indophenol coloration. In all regions the mucosa colored soonest and most deeply while the muscular layer and the submucosa assumed a relatively slight stain. Thus he concludes that the mucosa in both stomach and intestine possesses intense oxidative properties.

The fact that pepsin and trypsin are easily oxidized and that the mucosa of the stomach and intestine possesses intense oxidative properties would seem to offer an explanation of the fact that these organs are not digested by the pepsin and trypsin contained within their lumen. The assumption would be that the pepsin and trypsin immediately in contact with the mucosa of the stomach and intestine respectively undergo oxidation and that by such means the cells maintain their integrity during life.

Salkowski and others [1] have shown that all the body tissues possess the power of undergoing autolysis after death and that under certain normal as well as pathological conditions tissues and even organs may undergo autolysis during life. The atrophy of the thymus and the involution of the puerpural uterus might be mentioned as examples of normal auto-digestion. Various theories have been advanced to account for the fact that the tissues do not undergo auto-digestion during life as after death. One theory [2] is that there are in the living tissues anti-substances which hold the autolytic enzymes in check. Another theory [3] suggests that the tissues are protected by their alkaline reaction as it has been shown that an acid reaction is necessary for the activity of autolytic enzymes. A third theory assumes that the enzymes exist in a zymogen form and are activated or inactivated as the need may arise.

In view of the fact that autolytic enzymes in common with all

[1] Salkowski: Deutsche Klinik, 1903 (11), 147.
[2] Glaessner: Hofmeister's Beiträge, 1904, 4, 79.
[3] Wiener: Centralblatt für Physiologie, 1905, 19, 349.

the ordinary enzymes are destroyed by nascent oxygen an additional theory may be advanced, namely that the tissues maintain their integrity during life by means of their oxidative properties. This theory would assume that normally a balance exists between the autolytic enzymes and the oxidative processes of the tissues. It is known that in infectious diseases,[1] in diseases of the circulatory and respiratory systems,[2] in acute yellow atrophy of the liver and in chloroform and phosphorus poisoning autolysis may be increased to a marked degree. Without the oxygen continually supplied by the circulatory and respiratory systems oxidation in the tissues would be impossible. Since this is true, any special interference with either of these systems would presumably result in a decreased oxidation in the tissues. If the balance which has been assumed to exist between the oxidative and autolytic processes exists, then any interference with the supply of oxygen to the tissues should express itself in an increased rate of autolysis. Schlesinger [2] noted an intense self-digesting tendency of the tissues in diseases of the circulatory and respiratory systems. Under such conditions the amount of oxygen supplied to the tissues is decreased and the fact that under these conditions autolysis is increased would seem to support the above assumption. Jacoby [3] showed that the livers of dogs dead of phosphorus poisoning underwent autolysis more rapidly than normal livers, while Welsch [4] and Riess [5] found that oxidation in cases of this poisoning is decreased. Welsch made a study of the respiratory exchange in cases of phosphorus poisoning and found that the oxidative processes were decreased by about 20%. Riess proved the deficiency of oxidation also by showing the presence in the urine of large amounts of organic acids which are oxidized under normal conditions.

[1] Flexner: University of Pennsylvania Bulletin, July, 1903.

[2] Schlesinger: Hofmeister's Beiträge, 1904, 4, 87.

[3] Jacoby: Zeitschrift für physiologische Chemie, 1900, 30, 174.

[4] Welsch: Archives internationales de pharmacodinamie et de Thérapie, 1905, 14, 211.

[5] Riess: Berliner klinische Wochenschrift, 1905 (42), 44a, 54.

Conclusions

1. The facts that pepsin and trypsin are oxidized by nascent oxygen and that the mucosa of the stomach and intestine possesses intense oxidative properties may be used to explain the protection of these organs from self-digestion during life.

2. The fact that disatase is destroyed by nascent oxygen offers an explanation of the observation that the amount of this enzyme is decreased during the day and increased during the night in plants.

3. The fact that the autolytic enzymes are destroyed by nascent oxygen and that the tissues possess oxidative properties would seem to justify the assumption that normally there is a balance between the oxidative and autolytic processes in the living tissues. The fact that in certain pathological conditions where autolysis is increased the oxidative processes are decreased is in accord with this assumption.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XIV. The Influence of Smoking and of Pressure on the Abdomen (Constriction of the Belt) on the Gastric Hunger Contractions

By A. J. CARLSON and J. H. LEWIS

[*From the Hull Physiological Laboratory of the University of Chicago*]

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IT is generally held to be true that smoking shortly before a meal leads to depression of hunger and appetite. It is also a common belief that strong pressure on the abdomen ("tightening the belt") decreases or relieves the hunger sensation, at least temporarily. We are now in position to test the correctness of these beliefs by decisive experiments, at least as regards the influence of these measures on the objective hunger contractions and the subjective hunger sensations.

I. The Influence of Smoking on the Hunger Contractions and on the Hunger Sensations

Depression or inhibition of hunger by smoking is rendered probable by the fact that anything which stimulates the sensory nerve endings in the mouth and in the gastric mucosa inhibits the gastric hunger contractions in direct proportion to the intensity of the stimulation.[1] Smoking stimulates the nerve ending in the mouth in varying degrees according to the kind of tobacco used. Smoking frequently involves stimulation of nerve endings in the gastric mucosa owing to the swallowing of saliva containing nicotin, oils, tannic acid, and probably other irritating substances. Smoking may also act on the hunger mechanism in a third way, that is, through absorption of nicotin and other products of the combus-

[1] Carlson: This journal, 1913, xxxi, p. 212.

tion. This third possibility has not been investigated. It is well established, however, that even small quantities of nicotin in the blood leads to nausea and vomiting. Nausea and vomiting are accompanied by atony of the gastric fundus, which insures absence of hunger contractions and hunger sensations.

The effects of smoking on the gastric hunger contractions were first studied on Mr. V., our young man with the permanent gastric fistula. In his case smoking (cigars) leads invariably to inhibition of the hunger contractions. This fact was briefly reported in our first communication.[1] But Mr. V. is not an habitual smoker. It is therefore possible that the results obtained on him were simply due to the condition of nausea or disgust that smoking usually produces in the novice and hence not applicable to persons used to smoking.

The tests have now been repeated on several habitual smokers. *In so far as smoking influences the gastric hunger contractions this*

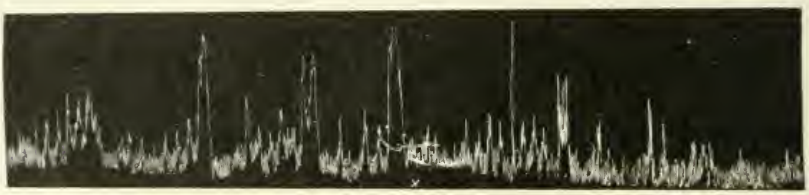

FIGURE 1. Tracing from the empty stomach of A. J. C. Bromoform manometer. Beginning of a period of hunger contractions, *x*, starting to smoke a "strong" cigar. Showing inhibition of the gastric hunger contractions and tonus.

influence is in the direction of inhibition. This inhibition appears to depend on the intensity of stimulation of the nerve endings in the mouth, a cigarette or "mild" cigar causing only slight inhibition, while a "strong" cigar or pipe causes complete and prolonged inhibition even when the gastric hunger contractions are at their maximum. A typical tracing showing this inhibition from smoking is reproduced in Figure 1.

If the cigar or pipe causes very strong stimulation of the nerve endings in the mouth, the inhibition of the hunger contractions may continue from five to fifteen minutes after the cessation of the stimulation. Thus even a brief period of smoking may suppress an entire hunger period.

[1] CARLSON: This journal, 1913, xxxi, p. 151.

The subjective sensation of hunger is diminished or abolished parallel with the gastric hunger contractions. But it seems to the authors that even a "mild" smoke diminishes the sensation of hunger rather more than one might infer from the slight depression of the contractions. This is probably due to the deviation of attention, the smoking acting partly as a "counter irritant."

Smoking inhibits the gastric hunger contractions. It is practically certain even in the absence of direct experiments that moderate smoking does not inhibit the gastric movements of digestion. The reason for the difference in the action of the same condition on the empty and on the filled stomach is not clear from present data.

II. The Influence of Constriction of the Belt

The experiments with constriction of the belt were made on three normal men. Mr. V. was not used in these tests for the

Figure 2. Tracing from the empty stomach of A. J. C. Beginning of a period of hunger contractions, *x*, strong pressure on the abdomen by belt. Showing inhibition of gastric hunger contractions of moderate strength by strong pressure on the abdomen by the belt.

reason that any considerable compression of the abdomen leads to pain and discomfort from pressure of the rubber tube in the gastric fistula. The tests were made with the subject standing up, sitting, and lying on the back, and at all stages of the gastric hunger contractions.

1. Strong contraction of the abdominal belt leads nearly always to inhibition of the gastric hunger contractions of weak or moderate strength, lasting from five to fifteen minutes. The inhibition may

be partial or complete, but in either case the hunger contractions reappear despite the continued pressure of the belt. This inhibition is obtained even when the belt constriction is moderate so that no discomfort or pain is produced. A typical tracing showing complete inhibition of the feeble hunger contractions on constriction of the belt is reproduced in Figure 2.

2. When the gastric hunger contractions are strong (the middle of a hunger period), constriction of the belt never causes complete inhibition. But so far as the increased abdominal pressure affects the hunger contractions the influence is in the direction of inhibition. The individual hunger contractions are weakened without

FIGURE 3. Tracing of the empty stomach of A. J. C. towards the end of a period of strong hunger contractions; *x-x*, strong pressure on the abdomen by belt. Showing a slight inhibition of the gastric hunger contractions during the compression of the abdomen. The hunger pangs were less intense during the time the belt was constricted.

suffering much change in the rate. Frequently, however, even a belt constriction that caused considerable discomfort has practically no influence on the hunger contractions, particularly if the subject is lying down. A tracing showing this slight inhibition is reproduced in Figure 3.

3. When the gastric hunger contractions are at their maximum in rate and amplitude, as is ordinarily the case near the end of a hunger period, no amount of belt constriction seems to influence the contractions. When this stage of the hunger period is reached the hunger pangs run their normal course in the presence of even painful belt pressures. A record illustrating this condition is reproduced in Figure 4.

4. All three subjects agreed that the belt constriction appeared to diminish or interfere with the hunger sensation to a greater

extent than seemed warranted from its effect on the hunger contractions. Several factors are probably involved in this discrepancy. (1) The belt constriction distracts the attention from the hunger impulses by stimulation of cutaneous nerves as well as by stimulation of nerve endings in the viscera, especially those of the peritoneum. (2) Strong pressure on the abdomen from without

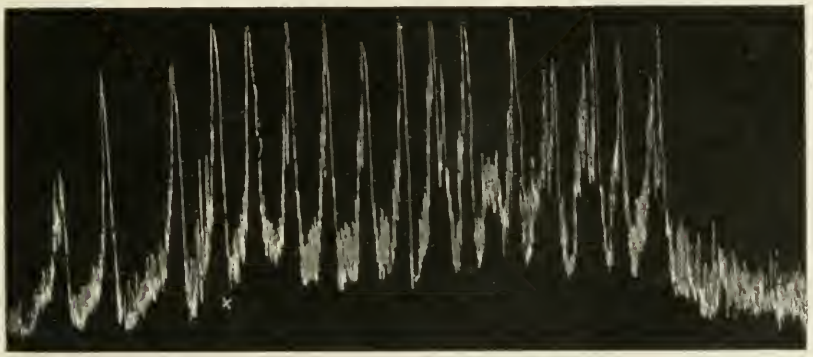

FIGURE 4. Tracing from the empty stomach of J. H. L. (standing position). *X*, strong pressure on the abdomen by belt. Showing completion of the hunger period despite the belt constriction. But the period does not culminate in the incomplete tetanus characteristic for Mr. L.

appears to induce, temporarily, a condition simulating in a feeble way the complex sensation of satiety.[1]

[1] According to R. Lennhoff (quoted in Jour. Amer. Med. Assoc., 1913, lx, p. 41) hunger and appetite are appeased with a less quantity of food when the belt is constricted than when the intra-abdominal pressure is regulated solely by the tonus of the abdominal muscles. Lennhoff ascribes this to depression of hunger and appetite by the pressure of the belt. Lennhoff's observation is probably correct, but his explanation erroneous. The actual hunger contractions and the hunger sensations are stopped by the first few morsels of food swallowed, while this may actually increase the appetite through stimulation of nerve endings in the mouth and in the mucus membrane of the oesophagus and stomach. This appetite sensation is gradually counteracted by the sensation complex of satiety, which depends in part on the distention of the stomach with corresponding readjustment of the tonus of the abdominal muscles. This feeling of fullness, which appears to be referred to the abdomen as a whole, is probably developed with less intake of food when the abdominal wall is mechanically prevented from relaxing owing to the pressure of the belt.

5. We have practically nothing but conjectures to offer in way of explanation of the mechanisms involved in the above inhibition of the gastric hunger contractions by strong pressure on the abdomen. Strong pressure on the abdomen causes temporary inhibition of the gastric hunger contractions in dogs, but the manipulation greatly disturbs the dogs, and disturbance from any cause leads to a temporary inhibition of the empty stomach in dogs with the splanchnic nerves intact. In dogs with the splanchnic nerves sectioned on both sides, strong pressure on the abdomen causes no distinct inhibition of the gastric hunger contractions. This points to the conclusion that belt constriction causes gastric inhibition, not by direct pressure on the stomach, but by direct stimulation of inhibitory nerves, or by mechanical (or sympathetic) stimulation of the adrenal glands, but through long reflexes. Belt constriction involves stimulation of cutaneous nerve endings, but the stimulation of the tactile nerve endings in the skin alone does not lead to this inhibition. The afferent path of the reflex must therefore involve abdominal proprioceptors. The splanchnic nerves probably constitute the efferent path of the reflex. We do not wish to be understood as denying the existence of local inhibitory mechanism that may be stimulated by mechanical manipulation of the abdominal organs. But our results indicate that strong belt constriction is not a sufficient stimulus for such local mechanisms. In any event, belt constriction is not a very efficient control of the hunger mechanism. A "strong" cigar is more efficient in that direction than a good belt.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XV. The Nervous Control of the Gastric Hunger Mechanism (Man, Dog)

By A. J. CARLSON
Assisted in the Experiments by J. H. Lewis and S. J. Orr
[*From the Hull Physiological Laboratory of the University of Chicago*]
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THE activity of the gastric hunger mechanism is subject to reflex (nervous), and to chemical or "hormone," control. The present paper deals with the nervous control. The results of our studies of the control of the hunger mechanism through substances in the blood will be reported later.

Some data on the side of nervous control of the gastric hunger mechanism have already been reported. It has been shown that the nervous mechanism involves both local centres (Auerbach's plexus in the stomach wall) and the central nervous system. Stimulation of the sensory nerves in the mouth, oesophagus and stomach mucosa inhibits the hunger mechanism by way of the splanchnic nerves as well as through the Auerbach plexus. It has also been shown that practically all stimuli that act on the gastric hunger mechanism via the central nervous system cause inhibition mainly through the splanchnic nerves. This is true, for example, in the case of the sight or smell of food on the part of dogs in hunger.

We now ask the reader's attention to the question of reflex control of vagus tone so far as this affects the stomach. We have determined the influence of the factors or conditions that are associated with lowering of the tonus of the central nervous system and the skeletal muscles, such as sleep, stimulation of the cutaneous nerve endings for heat, excessive muscular activity, as well as the factors that increase the skeletal neuromuscular tonus such as

stimulation of the cold nerve endings of the skin, moderate muscular activity, seeing and smelling palatable food, etc. These factors and conditions have been tested both on man and on dogs. Some of these conditions probably involve both chemical and nervous factors. Muscular activity may augment the gastric hunger activity by increasing the vagus tonus as well as by chemical changes in the blood. The same may apply to stimulation of the cold nerve endings of the skin. However, it is probable that if these conditions cause increase in the vagus tonus reflexly this response is more prompt than that induced by the changes in the blood following the increase or decrease in body metabolism due to stimulation. It is generally recognized that exercise, cold climate, and cold baths increase appetite and hunger. It does not follow that these conditions actually augment the gastric hunger contractions. The increase in hunger and appetite may be only apparent, that is, a condition of increased excitability of parts of the central nervous system, so that the afferent impulses that give rise to the sensations of hunger and appetite produce a greater central effect. If the gastric hunger contractions are actually increased, this may be due to changes in the blood rather than to increased vagus tonus.

It is well known that exposure of the skin to cold (as by bathing in ice water) may induce contracture or "cramps" of the digestive tract. This is especially the case during the height of gastric digestion. These cramps or contractures may be the result of circulatory disturbances or of changes in the blood rather than a direct reflex effect. Central processes are also able to induce contraction of the large intestine and the rectum, as shown by involuntary defecation in cases of great anxiety or fear.

From the point of view of biological adaptation we might expect the vago-gastric tonus to be directly affected by voluntary muscular activity and by exposure to cold, since both conditions involve increased oxidation and consequently increased need of food.

Experimental Procedure

Dogs. — Dogs with simple gastric fistulas were trained to run in a treadmill. When trained to run without much urging or

interference, records were taken of the contractions of the empty stomach so as to determine (1) whether muscular activity induces hunger contractions in the quiescent stomach, and (2) whether muscular activity augments the hunger contractions of an active stomach.

The hunger contractions of the stomachs of dogs were recorded for 2 to 4 hours, after a day's fast, the dogs being taken direct from the kennel without being exercised. On other days the same dogs were taken out for a 4 to 6 mile brisk walk before the 2 to 4 hour recording period.

Records of the gastric hunger contractions were taken with the dog lying quietly in the lap of an assistant. Then the body of the dog was surrounded with an ice pack, or the dog placed directly

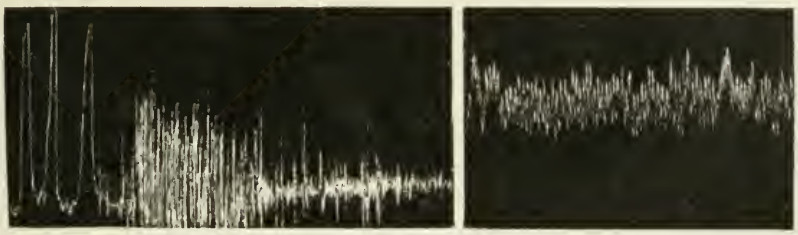


FIGURE 1. Tracing from the empty stomach of the dog. Bromoform manometer. *A*, dog standing in treadmill, stomach showing Type I hunger contractions. At *x*, the dog begins to run in the mill with the result that gastric hunger contractions are promptly inhibited. The running was kept up for 60 minutes. *B*, record from stomach of the same dog 45 minutes after he ceased running, showing increased gastric tonus and Type III hunger contractions.

on a slab of ice. After some training the dogs do not appear much disturbed by the ice pack or the slab of ice. The ice pack was applied with the stomach quiescent as well as in hunger activity.

All of the above procedures were used on normal dogs and on dogs with the splanchnic nerves sectioned on both sides, in order to have the tonus fibres of the vagi unopposed by the splanchnic inhibitory influence.

Man. — The tests were made on the author, on Mr. V. (the gastric fistula case), and on three assistants (J. H. L., S. J. O., A. M. P.).

Records were taken of the gastric tonus and hunger contractions with the man standing, and walking or running in place. Tests were also made after muscular exercise (playing tennis, walking 6 to 12 miles).

The influence of exposure to cold on the gastric hunger mechanism was tested in the following way. (1) While records of the gastric tonus and hunger contractions were being taken, the man, stripped of his clothes, was subjected to cold or warm showers for varying periods. The cold showers were at times sufficiently cold or prolonged to cause intense shivering. (2) The man stripped of his clothes in a cold room was covered up on a couch so as to feel comfortably warm. At the desired moment in the gastric activity, that is, during a period of quiescence or in the midst of a period of hunger contractions, the covers were removed and the cold air of the room set in motion by a fan placed close to the person. This brought on shivering in a few minutes. (3) The man arose at 7 A. M. and, without the usual cold bath and breakfast, proceeded to the laboratory and records of the gastric tonus and hunger contractions were taken from 8 to 12 A. M. These served as controls. On other days the man arose at 6 A. M., took a cold bath (this was prolonged until the discomfort became very severe), followed by a brisk walk, when records were taken from 8 to 12 A. M.

Results on Dogs

1. Effects of running in treadmill. — The initial effect on gastric tonus and hunger contractions of running in the mill is always in the direction of inhibition — usually complete inhibition, and if the dog is started running in the midst of a period of gastric quiescence there is no evidence of increased gastric tonus or beginning hunger contractions. If the dog is made to run at high speed the inhibition persists during the entire period even if the running is kept up for one to two hours. When the dogs ran at rather high speed for an hour or more the gastric inhibition usually persisted from 20 to 40 minutes after the dogs stopped running. The return of gastric tonus and hunger contractions in such cases is very gradual. But frequently when the gastric tonus finally recovered after a running period it was higher than before the dogs began to run.

Thus a dog showing Type I or II hunger contractions when he started to run in the mill may show an increased tonus and Type III hunger contractions 30 minutes after he stopped running, while the running period itself was accompanied by complete gastric inhibition. If the dog runs only moderately fast in the mill the gastric tonus and hunger contractions reappear during the running period, or come on during the running, in case the dog is started when the empty stomach is quiescent. A typical tracing showing this gastric hunger inhibition synchronous with running with subsequent recovery to greater gastric tonus is reproduced in Figure 1.

These facts indicate that the carnivorous animal in pursuit of its prey must be urged on by something else than the pangs of hunger, as these are inhibited by the chase.

2. Effects of 4–6 mile walk. — Eight tests (with a corresponding number of controls) on two dogs failed to show any marked effect of a 4 to 6 mile walk on the gastric hunger contractions either in the way of increase or decrease, the records being taken during the two hours following the walk. These walks certainly caused no depression of the dog's hunger contractions. But the dog that showed Type II contractions in the control usually showed Type II contractions after the walk with no definite increase either in rate or intensity. This should be noted, however, that after these walks both dogs showed greater restlessness than when taken from the kennels directly to the laboratory. They were not so easily quieted in the lap of the assistant. This rather restless condition of the dogs may have counteracted any augmentation of gastric hunger contraction due to the walk, as restlessness from any cause tends in the dog to inhibit the hunger contractions.

3. The effect of intense stimulation of the cutaneous nerve endings for the sensation of cold. — When a dog is lying quietly and comfortably in the lap of an assistant, surrounding the dog with an ice pack or placing him directly on a slab of ice leads to struggling and restlessness. After a number of repetitions of these procedures most dogs become so accustomed to it that they pay little or no attention to the change and show no restlessness or struggling. If the dog is disturbed or struggles when placed on a slab of ice or surrounded by an ice pack there always follows a temporary inhibition of gastric tonus and hunger contractions.

But this does not indicate the initial or primary effect of stimulation of the cutaneous nerve endings for cold, because the same type of inhibition is induced by restlessness or struggle for any cause. After the dog is trained to these procedures strong stimulation of the cutaneous nerve endings for cold by the ice pack, by placing the dog on a slab of ice, or by turning on an electric fan in a cold room after uncovering the dog, has no immediate effect on the gastric tonus and hunger contractions. There is usually an

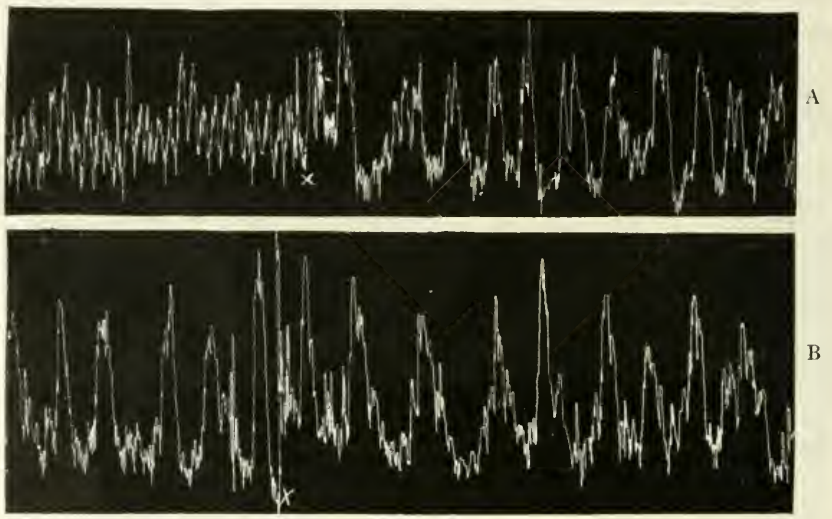


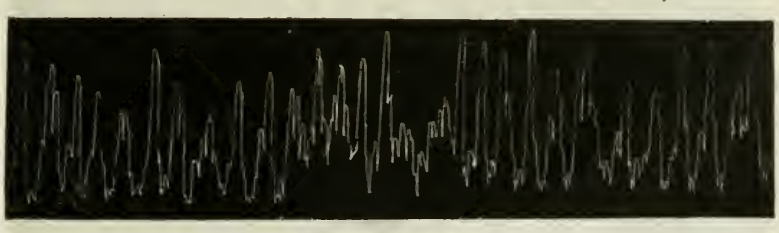
FIGURE 2. Tracings from the empty stomach of dogs. Bromoform manometer. *A*, dog covered with an ice pack for 30 minutes and shivering. Stomach shows Type III hunger contractions. At *x*, the ice pack is removed, and the stomach promptly passes into Type II hunger contractions. *B*, at *x* the dog was placed on a slab of ice. Showing no immediate effect on the gastric hunger contractions, despite the shivering of the dog.

increase in the intra-abdominal pressure owing to the increased tonus of the abdominal muscles. If the ice pack is applied during a period of gastric quiescence there is no immediate increase in gastric tonus or initiation of the hunger contractions, even though the dog starts to shiver violently in a few minutes. If the ice pack is applied during the hunger contractions, these contractions do not change appreciably either in rate or strength, at least for some time. This is true even when the dog shivers considerably. It would thus seem that the vagus centres governing the gastric

tonus are not directly affected by even very strong stimulation of the cutaneous nerve endings for cold.

In several instances the continued application of the ice pack (30 to 40 minutes) and in consequence continued shivering lead to a gradually increased gastric tonus and the appearance of Type III hunger contractions. These may be due to changes in the blood as a result of increased oxidation, or they may appear from causes not connected with the stimulation of the cold nerve endings. Such change in the hunger contractions is not infrequent in dogs, even when they are lying undisturbed and comfortable in the lap of an assistant.

In two cases the Type III hunger contractions changed to Type II on removal of the ice pack (Figure 2A). The change



X

FIGURE 3. Tracing from the empty stomach of dog with section of splanchnic nerves on both sides. At *x* the dog is surrounded by an ice pack. Showing no effect on the hunger contraction although the ice pack caused the dog to shiver.

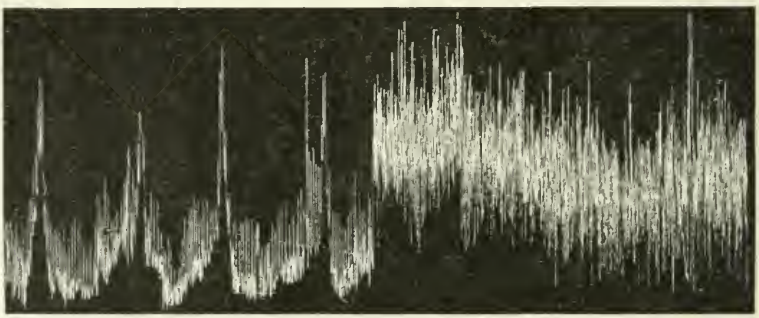
came on promptly on removing the ice pack. I am inclined to attribute this change to some shifting of the position of the stomach or shifting of the position of the balloon in the stomach as the result of the removal of the pressure of the ice pack over the abdomen, rather than as a reflex effect.

It is conceivable that the stimulation of the cold nerve endings in the skin does influence the vago-gastric tonus centres, but the stimulation acts equally on the gastric inhibitory mechanism via the splanchnic nerves so that the net result on the empty stomach is nil. This possibility is cleared up by the tests on dogs with section of both splanchnic nerves. Tests were made on two dogs on which this operation had been performed. The results were practically identical with those on normal dogs. The ice pack neither decreased nor increased the gastric hunger contractions

(Figure 3). It is therefore clear that the nervous impulses that give rise to the sensation of cold and induce increased neuro-muscular tonus in general have no direct action on the vago-gastric tonus centres.

Results on Man

1. The direct effect of muscular exercise. — Standing or walking in place has no effect on the gastric tonus or hunger contractions. But running in place promptly inhibits the hunger contractions (Figure 4). The degree and duration of the inhibition is on the whole directly proportional to the speed of the running.



X

Figure 4. Tracing from the empty stomach of man (A. J. C.) in standing position. Beginning of a hunger period. At *x* the man began running, with the result that the hunger contractions were promptly inhibited.

In some cases walking seemed to prolong a hunger period without changing the rate or intensity of the individual contractions. In no case did walking or running induce hunger contractions in the quiescent stomach. The results on man are thus identical with the results on dogs. In both species rapid running is accompanied by inhibition of the gastric tonus and hunger contractions. In the case of the dog running in the treadmill, one cannot be sure that the exercise is strictly voluntary and enjoyable. The inhibition may therefore be due to certain emotional states (anxiety, discomfort, mild anger or fear). This possibility is eliminated by the tests on man. In the men the conditions of the emotions when running in place were not different from that when standing or

walking in place. In no case was the running carried to the point of respiratory, cardiac, or muscular distress.

2. The after effects of muscular exercise. — Moderate exercise in the form of playing tennis or walking four to eight miles was taken in the afternoon. No supper was taken, and the motor condition of the empty stomach was recorded from 8 to 12 P. M. The tracings obtained on the days specific exercise was taken show on the whole greater gastric hunger activity than the controls. The periods of quiescence become shorter. This tends to make the gastric hunger contractions more or less continuous, and there appears to be some increase in the rate of the contractions. A typical experiment (S. J. O.) may be cited in the way of illustration.

Record of control day. — Lunch 1: 30 P.M. No special exercise. No supper. Period of observation 8 to 12 P.M.

8 to 10 P.M. Stomach practically quiescent.

10 to 10: 40. Strong hunger contractions ending in tetanus.

10: 40 to 11: 35. Stomach quiescent.

11: 35 to 12: 05. Moderate hunger contractions ending in tetanus.

Record of exercise day. — Lunch 1: 30 P.M. No supper, tennis 4 to 5 P.M.; walking 6 to 7 P.M. Period of observation 8 to 12 P.M.

8: 15 to 9: 50. Practically continuous hunger contraction ending in strong tetanus.

9: 50 to 10: 20. Stomach quiescent.

10: 20 to 11: 40. Strong hunger contractions ending in tetanus.

Total duration of hunger periods from 8 to 12 P.M. { Control day; 70 minutes. / Exercise day; 190 minutes.

In some instances there was no marked difference between records of the control and the exercise days. This is to be expected since the activity of the gastric hunger mechanism depends in part on factors not understood or controlled. Exercise that brings on a degree of fatigue bordering on exhaustion seems to depress the gastric hunger mechanism. But our experiments on this point are as yet too few to permit a final conclusion.

3. The direct effect of stimulation of the cold nerve endings of the skin. — The immediate effect of stimulation of the cold nerve endings of the skin by ice pack, alcohol bath, cold shower bath, or

cooled air is inhibition of the gastric tonus and hunger contractions (Figure 5), and the degree of inhibition is proportional to the intensity of the stimulation. In no instance did we observe an initial increase in gastric tonus and hunger contractions. When the stimulation is continued the inhibitory effects gradually diminish even though the man shivers intensely from the cold. In this way the gastric hunger contractions may return to their normal rate, intensity and regularity, while the man is shivering and jerk-

FIGURE 5. Tracing from the empty stomach of man (A. J. C.) in the midst of a period of hunger contractions. The man was stripped and covered up with blankets in a cold room (20° C.). At x the covers were removed and a fan close to the man started. Shivering began at x^1. Showing a temporary but partial inhibition of the hunger contractions.

ing like a dog in mild parathyroid tetany. It may be noted in this connection that mild, and in some instances fairly severe, parathyroid tetany in dogs does not appreciably influence the gastric hunger contractions.[1]

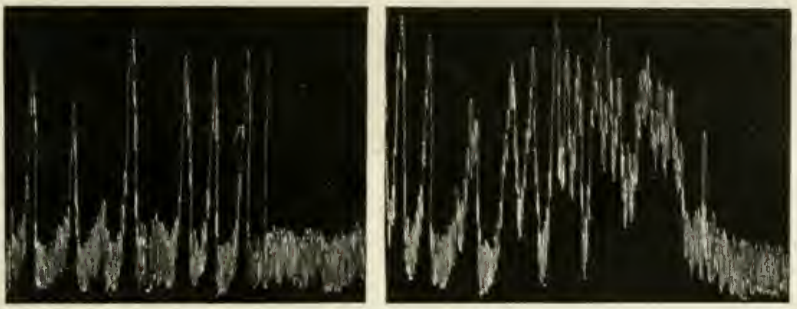
Intense stimulation of the heat nerve endings of the skin (hot shower) produces practically the same initial inhibition as the corresponding stimulation of the cold nerve endings.

While it is true that on prolonged stimulation of the cold nerve endings of the skin during a period of gastric hunger contractions, the inhibitory effects gradually disappear so that the contractions reappear in their normal intensity, these contractions are always felt as weaker than the normal, or may not be felt at all. Evidently the intense sensation of cold dominates consciousness to the exclusion of the gastric hunger pangs.

It is well known that strong stimulation of the cold nerve

[1] CARLSON: This journal, 1913, xxxii, p. 397.

endings of the skin causes a reflex increase of tonus of the urinary bladder. In several instances we started these stomach tests on the men at a time when their bladder was known to contain 50 to 200 cc. of urine. This permitted us to compare the reflex effect of cold on the stomach and on the bladder tonus without a balloon in the bladder. When the cold stimulation began during a period of gastric quiescence and was continued long enough to induce intense shivering a strong desire to micturate soon developed while there was no evidence of increased gastric tonus. Prolonged cold stimulation may produce so great tonus of the bladder that micturition cannot voluntarily be inhibited. The tonus centres



A B

FIGURE 6. Records of culminations of periods of gastric hunger contractions of A. J. C. *A*, the ordinary type of ending of the hunger periods without tetanus. *B*, hunger period ending in incomplete tetanus three hours after intense stimulation of the cold nerve endings (bath at 10° C. for 15 minutes).

of the urinary bladder are, the vago-gastric tonus centres are not, directly influenced by cold stimulation of the skin.

When the cold nerve endings of the skin are stimulated, as above, during a period of quiescence of the empty stomach, the stomach remains quiescent. If there is any change in the gastric tonus it is in the direction of inhibition. Nevertheless, this cold stimulation, if not sufficiently intense to be painful, seemed to induce a "sensation of emptiness" in the abdominal region, a sensation that seemed to be associated with appetite and desire for food. We record this with some hesitation, for this sensation of emptiness may be purely subjective (autosuggestion). It may also be due to the increased tonus of the abdominal muscles. In any event, this sensation is clearly different from the hunger pangs.

4. The after effect of stimulation of the cold nerve endings of the skin.—All of the tests in this group were made on one man (A. J. C.). A prolonged cold bath 6 to 7 A. M. followed by a brisk walk nearly always resulted in increased hunger activity of the stomach as recorded for the period 8 to 12 A. M. (Figure 6). The temperature of the water varied from 5° C to 15° C. The subject remained in the water as long as was deemed safe (10 to 20 minutes), despite discomfort and pain. Water at this temperature soon brings on shivering, contracture and at times severe headache, and it requires much vigorous exercise to restore the feeling of warmth. Rubbing the skin (rough towel) seems to be of no aid.

A typical experiment may be cited in illustration.

Control record. — No bath or breakfast. Observation period 8 to 12 A.M.

8: 50 to 10. 26 fairly strong hunger contractions; no tetanus.

11: 00 to 11: 45. 22 fairly strong hunger contractions; no tetanus. Gastric tonus on the average 5 cm. Bromoform.

Test period. — 6 to 6: 15 A.M. cold bath (temp. of water 10° C.). No breakfast. Observation period 8 to 12 A.M.

8 to 9. 32 strong contractions; no tetanus.

9: 45 to 10: 25. 23 fairly strong contractions; no tetanus.

11: 15 to 11: 45. 19 strong contractions ending in tetanus. Gastric tonus on the average 8 cm. Bromoform.

Control period. — 48 hunger contractions; no tetanus.

Test period. — 74 hunger contractions; tetanus.

Under ordinary conditions the periods of gastric hunger contractions of the author do not end in tetanus, but the hunger tetanus appears after 3 to 4 days' complete starvation.[1] Fifteen to thirty minutes' intense stimulation of the cold nerve endings thus seem to bring about a condition similar to prolonged starvation. This is in harmony with the observation of Lusk that such stimulation quickly renders the liver free from glycogen.[2] This effect of cold on the gastric hunger mechanism is obviously an indirect one, or through changes in the blood, and not a direct reflex from the skin.

[1] Carlson: This journal, 1914, xxxiii, p. 95.

[2] Lusk: This journal, 1911, xxviii, p. 427.

Lusk has shown that intense cold leads to quicker and more complete oxidation of the body glycogen than prolonged starvation. And it is interesting to note that the same stimulus causes not only an increase in the *gastric hunger contractions*, but also an even greater increase in the subjective hunger and appetite sensations, probably owing to an increased excitability of the central nervous system. The increased desire to eat after a cold bath, in the case of the healthy individual, is a universal experience. I have investigated this matter in the case of young children, with whom habit or intelligence cannot be assigned as the cause for seeking food after a cold bath. It was found that young children react in the same way as adults.

5. The gastric hunger contractions during sleep. — During sleep there is decreased activity of the central nervous system in general, decreased tonus of the skeletal muscles, decreased tonus of the musculature of the blood vessels, at least in certain parts of the vascular system, decreased tonus of the urinary bladder, etc.; in short, a lowered activity of all the neuro-muscular mechanisms so far investigated. One might have expected that in so far as the tonus of the empty stomach depends on a central influence by way of the vagi, the gastric tonus and hunger contractions should be diminished during sleep. But instead of being depressed in sleep the hunger contractions continue with the same vigor as during the waking state, and in many instances with increased vigor. This has been established both for man and dog and is reported in previous communications.[1] We have nothing new to add on this point, except the mere confirmation of the facts already published. It is referred to in this connection because of its bearing on the question before us, the control of the vago-gastric tonus mechanism. The increase in the gastric hunger contractions during sleep may be due to elimination of all inhibitory impulses via the splanchnic nerves. But the absence of depression certainly indicates that the vago-gastric tonus mechanism, at least in man and dog, occupies a unique position in the organism, a degree of independence of afferent impulses (exteroceptors) and central processes not known in the case of any other neuro-muscular apparatus.

[1] Carlson: This journal, 1913, xxxii, p. 369; 1914, xxxiii, p. 95.

6. The effect of cerebral states (emotional states, Intellectual processes). — It has been shown in previous communications that in the dog the nervous processes of joy, fear, anger, eagerness (from food), attention, etc., cause temporary inhibition of the gastric hunger contractions. This inhibition takes place by way of the splanchnic nerves, not by a depression of the vagus tonus. This, again, points to an unusual independence of the vago-gastric tonus apparatus.

In man, intellectual processes (attention, reading, figuring, arguing) have no distinct influence on the course of the hunger periods. Actual anxiety causes temporary inhibition (probably through the splanchnics). We have not been in position to make observations on the effects of actual anger, fear and joy, but there is no reason to believe that these processes act differently in man from that in the dog. In man we have paid particular attention to the effects of seeing and smelling palatable food, as it seemed *a priori* reasonable that the impulses generated by these stimuli might make more intimate connection with the vago-gastric tonus apparatus. Extensive experiments on Mr. V. and a number of tests on the author seem to show that this is not the case. These stimuli neither initiate nor augment the gastric tonus and hunger contractions; so far as they influence them at all, it is in the direction of inhibition. One of the tests on the author might be given. Before beginning the five days' starvation period, our colleague, Dr. Luckhardt, was asked to bring in, unknown to the author, a tray of choice food in the midst of a hunger period. The arrangements being made, the matter was dismissed from the author's thoughts.

One o'clock on the morning of the fourth starvation day the subject was asleep and the record showed the midst of a period of vigorous and regular hunger contractions. He was awakened to behold Dr. Luckhardt and the assistant enjoying a feast of porter-house steak with onions, German fried potatoes, and a tomato salad. The tray with edibles was placed not more than four inches from the subject's face and the delicious odor of the food filled his nostrils. He felt the hunger pangs as unusually intense, and there was considerable salivation. However, the gastric hunger contractions were not increased either in rate or intensity.

In a few minutes, on the contrary, the hunger contractions became weaker and the intervals between them greater, and the period terminated by this gradual depression much sooner than it probably would have done in the absence of the dinner scene. This was undoubtedly due to local acid inhibition from copious secretion of appetite gastric juice.

Our data on normal men and dogs seem incapable of any other interpretation than that the vago-gastric tonus apparatus so far as it concerns the empty stomach occupies a unique and physiologically isolated position, in the way of nervous control, while the inhibitory apparatus via the splanchnic nerves is readily influenced by central and reflex processes. We feel, however, that these observations must be extended to other groups of vertebrates as well as to such pathological cases in man in which there are indications of abnormalities of the vago-gastric tonus, before final explanations are attempted or speculation indulged in as to the usefulness of this physiological isolation.

This evidence for the physiological isolation of the hunger mechanism in the way of positive central control is of interest in connection with the view that the cravings of hunger and appetite are subjective and largely a matter of habit, and that the periodicity or intensity of these cravings may be altered almost at the will of the individual. Chittenden[1] states this view as follows. "The so-called cravings of appetite are largely artificial and mainly the result of habit. Anyone with a little persistence can change his or her habits of life, change the whole order of cravings, thereby indicating that the latter are essentially artificial and have no necessary connection with the welfare or needs of the body. The man who for some reason deems it advisable to adopt two meals a day in place of three or four, at first experiences a certain amount of discomfort, but eventually the new habit becomes a part of the daily routine, and the man's life moves forward as before, with perfect comfort and without a suggestion of craving, or a pang of hunger."

Our studies of the hunger mechanism seem to show that the above view is essentially wrong. In the normal individual the gastric hunger periods begin as soon as the stomach is empty and

[1] CHITTENDEN: The Nutrition of Man, New York, 1907, p. 164.

continue (in the absence of inhibitory processes) as long as the stomach is empty, irrespective of the time of day or night, and without reference to the time the individual is accustomed to eat. In individuals accustomed to the usual three meals in daytime and to sleep during the night, the gastric hunger periods are more frequent and usually more vigorous during the night (in sleep) than during the day, provided, of course, the stomach is empty. In the normal individual the empty stomach exhibits periodic hunger activity, and there is no evidence to show that this primary automotism of the empty stomach is in the least influenced by eating one or by eating five meals a day. The basis for the view that the time of appearance of the "cravings of hunger" can be changed at will is probably to be sought in the fact that the milder hunger contractions do not enter consciousness as pangs of hunger if the individual's attention is directed into other channels. They are felt as hunger pangs if the individual's attention is directed towards food and eating. The attention is thus directed, consciously or sub-consciously, about the time the individual is accustomed to eat. The periodicity of this subjective attention to the milder hunger cravings can probably be altered by training. But this applies only to relatively mild pangs of hunger. The more severe "cravings of hunger" caused by the gastric hunger tetanus rise above the limen of consciousness, except in deep sleep or under conditions of cerebral process involving intense interest. When an individual who is used to eat three times a day turns to a régime of one meal a day, the quantity of food ingested in that one meal is much greater than that at any one of the three meals a day régime. The emptying of the stomach and the appearance of the pangs of hunger are correspondingly delayed. The view that prompt appearance and the persistence of the gastric hunger activity in the empty stomach have no relation to the actual need of the individual for food cannot be seriously maintained for the normal animal.

Summary

1. Moderate muscular activity (walking) has no direct influence on the gastric hunger mechanism. Intense muscular activity (running) inhibits the hunger mechanism in direct proportion

to the intensity and duration of the exercise. Feeble hunger contractions may continue during moderate running. There appears to be some increase in the gastric tonus and hunger contractions as an after effect of moderate exercise.

2. Stimulation of the cold nerve endings of the skin does not affect the vago-gastric tonus apparatus. If the stimulation is of sufficient intensity it induces (especially in man) a temporary inhibition of the gastric hunger contractions via the splanchnic nerves. A similar inhibition is induced by strong stimulation of the cutaneous nerve endings for warmth. There is a distinct increase in the gastric tonus and hunger contractions as an after effect of prolonged and intense stimulation of the cold nerve endings of the skin.

3. The vago-gastric tonus mechanism is not influenced by the condition of sleep, except in the way of augmentation, owing to the elimination of all inhibitory processes via the splanchnic nerves.

4. The vago-gastric tonus mechanism is not affected by intellectual processes or emotional states, except in so far as these cause inhibition of the gastric tonus and hunger contractions via the splanchnic nerves.

5. It is clear from the above that in normal individuals (man, dog) the vago-gastric tonus apparatus, at least so far as it concerns the empty stomach, is physiologically isolated from the exteroceptors and from many, if not all, central processes, while the splanchnic inhibitory apparatus is readily accessible to these processes. The biological significance of this exceptional and unique isolation of the tonus apparatus of the hunger mechanism probably lies in the importance of the hunger mechanism being regulated on its positive side primarily by the state of nutrition, that is, through the blood, rather than by the fleeting changes in the nervous system.

ADRENAL DEFICIENCY AND THE SYMPATHETIC NERVOUS SYSTEM

By R. G. HOSKINS and HOMER WHEELON

[*From the Laboratory of Physiology of the Northwestern University Medical School*]

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ELLIOTT'S final demonstration of the intimate relationship subsisting between epinephrin and the sympathetic nervous system [1] has seemed to many physiologists largely to have solved the problem of the functional significance of the adrenal glands. The fact that injecting epinephrin is exactly equivalent to a general stimulation of the sympathetic system and the common supposition that an animal after removal of its adrenals dies in a condition of vasomotor failure at least strongly suggest that the paramount function of these glands is to maintain tonus in the sympathetic system. This hypothesis may be designated the "tonus theory." It supposes that the adrenals constantly pour into the blood stream epinephrin in sufficient amount to keep the sympathetic system in a condition of partial stimulation.

A number of facts may be mentioned, however, which seem to disprove the theory. Blood pressure which is directly under sympathetic control may be taken as a criterion of sympathetic functioning. Experiments have shown that epinephrin at a sufficiently slow rate can be introduced continuously into a vein without producing any demonstrable effect.[2] If the rate is increased to an effective degree the result supposedly is either an increment of the influence of the epinephrin normally derived from the animal's own glands or else, if that has been in abeyance, of the appearance of the normal physiologic epinephrin effect. But the first apparent

[1] Elliott: Journal of Physiology, 1905, xxxii, p. 401.

[2] Hoskins and McClure: Archives of Internal Medicine, 1912, x, p. 343.

result of such injections is a *depression* of vascular tonus.[1] This is Elliott's "paradoxical reaction." [2] Whether, however, this be the paradox, is merely a matter of orientation; the pressor effect of larger doses might equally well be so designated. As a matter of fact a reversal of reaction as the acting quantity of epinephrin is increased seems characteristic. Such reversals have been observed in case of intestinal peristalsis,[3] uterine contractions,[4] pulmonary circulation[5] and general blood pressure[1]. It is the effects observed with the higher dilutions that are probably to be regarded as normal.

Another fact militating against the "tonus" theory is that sudden ligation of the adrenal circulation under conditions whereby nothing else is affected has absolutely no influence upon blood pressure until the lapse of a period far greater than that required for the destruction of any accumulated epinephrin that might have been present. The matter has been conclusively determined both in anaesthetized and in conscious animals.[6] If the theory were true a fall of pressure exactly commensurate with the preceding tonic influence would necessarily occur immediately after the ligatures were placed.

Possibly most significant is the fact that the injection of any quantity of epinephrin adequate to exert a minimal "tonic" influence upon blood pressure gives rise to conditions incompatible with ordinary existence. In dogs, such quantities produce complete paralysis of the gastrointestinal tract.[7] In rabbits also this

[1] Moore and Purington: Archiv für die gesamte Physiologie, 1900, lxxxi, p. 483. Hoskins and McClure: Archives of Internal Medicine, 1912, x, p. 353. Cannon and Lyman: This journal, 1913, xxxi, p. 376.

[2] Elliott: Journal of Physiology, 1912, lxiv, p. 402.

[3] Hoskins: This journal, 1912, xxix, p. 363.

[4] Stewart: Journal of Experimental Medicine, 1912, xv, p. 547.

[5] Desbouis et Langlois: Comptes rendus de la Société de Biologie, 1912, lxxii, p. 674.

[6] Hoskins and McClure: This journal, 1912, xxx, p. 192. Kahn: Archiv für die gesamte Physiologie, 1911, cxl, p. 216. Trendelenburg, W: Zeitschrift für Biologie, 1914, lxiii, p. 155.

[7] Hoskins and McClure: This journal, 1912, xxxi, p. 59.

is true of some individuals.[1] Moreover, during such injections a condition of glycosuria arises before a pressor effect appears.[2]

Attractive as the tonus theory is, such data render it no longer tenable. But the fact remains, — adrenal extirpation is fatal and the final symptoms are supposed to include a primary failure of functions under sympathetic control, — notably of blood pressure. Elliott[3] has offered the suggestion that adrenal deficiency results, not necessarily in the loss of any tonic stimulant, but of a substance necessary for the maintenance of sympathetic irritability; that is, that epinephrin is of importance in the metabolism of the sympathetic system or more particularly of the myoneural "receptive substance." There is nothing in the available evidence which disproves the suggestion.

Another hypothesis equally capable of explaining the results of adrenal extirpation has been made by Hoskins and McClure,[4] — namely that the adrenals in some way directly promote the metabolism of the muscular tissues. In the absence of the glands myasthenia develops. This asthenia, if it included the circulatory apparatus, would lead to low blood pressure. The negative phase of the hypothesis is equally tenable, that is, that the adrenals destroy some substance which interferes with muscular metabolism. In this form the suggestion is an old one.

Elliott's suggestion is amenable to experimental investigation. If adrenal destruction results in an interference with sympathetic functioning the fact should be easily demonstrable. Accordingly the experiments herein reported were undertaken.

At first thought, an animal in a late stage of fatal adrenal deficiency might seem to offer the most favorable conditions for determining the matter. But such can scarcely be the case. An animal in the final stages is simply moribund and can afford little definite evidence as to how it became so. The classic description of the effects of adrenal extirpation is not at all significant. It is nothing more than the description of an animal dying from any

[1] Trendelenburg, P., und Fleischhauer, K: Zeitschrift für die gesamte experimentelle Medizin, 1913, I, p. 393.

[2] Gramenitzki: Biochemische Zeitschrift, 1912, xlvi, p. 186.

[3] Elliott: Journal of Physiology, 1904, xxxi, p. xx.

[4] Hoskins and McClure: This journal, 1912, xxx, p. 195.

non-irritative slowly cumulative cause; muscular weakness, subnormal temperature, feeble respiration and pulse, low blood pressure — none of these are at all characteristic and any or all might be secondary effects. More significant are the conditions when the animal is first reacting to the operation. Primary effects alone are then in evidence. Our studies were made, therefore, mostly on earlier stages, from two to six hours after removal of the glands. In one instance, however, an interval of nine hours elapsed and one animal under urethane anaesthesia was kept under continuous observation from the time of operation until death, about ten hours later.

Various means of investigating the condition of the sympathetic system were contemplated but only three were found necessary. These are stimulation of afferent nerves, and injections of "adrenalin" and of nicotin. Blood pressure was used as a criterion of sympathetic conditions and by these three means conclusive evidence as to the conditions of the vasomotor reflex arc were secured before and after adrenal destruction. As a matter of fact the epinephrin, as the final results showed, might have been omitted, but this fact was not foreseen. We made observations in each case also with pituitrin but these added nothing significant to the evidence and are not reported.

In all the experiments dogs were used. The plan of procedure in most cases was to make a preliminary study of vasomotor conditions, remove the adrenals, and after the animal began to react to adrenal deficiency, make a second investigation of the vasomotor system. In some instances the removal of the adrenals immediately preceded the first determination. Under ether anaesthesia the animals were prepared for recording blood pressure. Into a femoral artery was inserted a Hall reservoir cannula [1] which was filled with 10% sodium citrate solution and connected directly with a recording mercury manometer. Into the contiguous vein was tied a large-bore cannula which was connected by rubber tube with a reservoir of 0.8 per cent sodium chloride solution suspended at a height of two feet. The effects of nicotin and of adrenalin vary materially according to the speed at which they are

[1] The Hall cannula is an ordinary glass arterial cannula in which is blown a 20 cc. reservoir.

introduced. To secure uniformity in this regard the following technique was employed: The substance was injected by a hypodermic syringe into the rubber tube just above the venous cannula. Its entrance into the cannula was prevented by a clip. Immediately then before diffusion occurred the clip was released and the drug instantaneously flushed into the vein. Previous investigation had shown that this technique gives results at different times which are comparable.[1]

In the leg not used for the cannulas a crural nerve was laid bare for stimulation. For this the nerve was simply picked up on platinum electrodes. In order to obviate local injury of the axones and the effects of variability of contact at different times, the electrodes during the time of stimulation were moved back and forth over a segment about two centimeters long. A strength of current was employed just sufficient to give a definite pressor response. The same strength of course was used in all experiments in any given animal. By these means blood pressure records were obtained showing the initial degree of vasomotor tonus and of vasomotor irritability. They gave an index also of the strength and rapidity of the heart beat. Some knowledge of respiratory conditions could be deduced from the respiratory waves of the pressure curves. At the conclusion of the determinations the vessels were tied off and the incisions in the legs closed with sutures. In the interim between experiments the animals were kept lightly under the influence of morphine. In the succeeding determinations the same blood vessels and nerves and the same cannulas were used as at first.

That complete ligation of the adrenal glands is equivalent to their actual extirpation has been shown by Mossou and Le Play [2] and confirmed by Allen.[3] Animals die with the same symptoms after either procedure. Ligation has the material advantage that it can be carried out in ten minutes and with a minimal degree of shock. We decided, therefore, to use this method of creating adrenal deficiency. In order to be certain of the absolute isolation

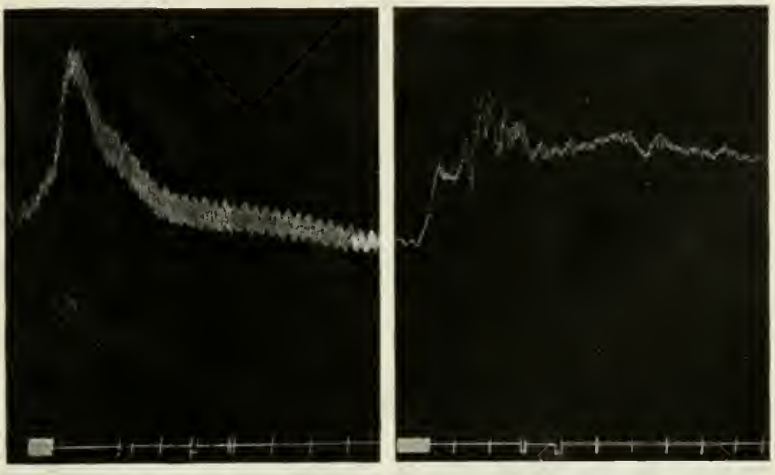
[1] Hoskins and Wheelon: This journal, 1914, xxxiv, p. 82.

[2] Mossou et Le Play: Comptes rendus de la Société de Biologie, 1909, p. 36, p. 83.

[3] Allen: Glycosuria and Diabetes: Boston, 1913, p. 863.

of the glands two ligatures were introduced together under each. One ligature was then tied tightly at the mesial, the other at the lateral side. All possible connection with the rest of the body was thereby destroyed. Our actual results show that diffusion from the isolated organs is not a factor. The animals promptly developed the characteristic signs of deficiency just as after actual extirpation.

Figure 1 shows the reactions of dog 51 to 0.4 cc. of 1:20,000 solution of "adrenalin" before and after adrenal ligation. The



A B

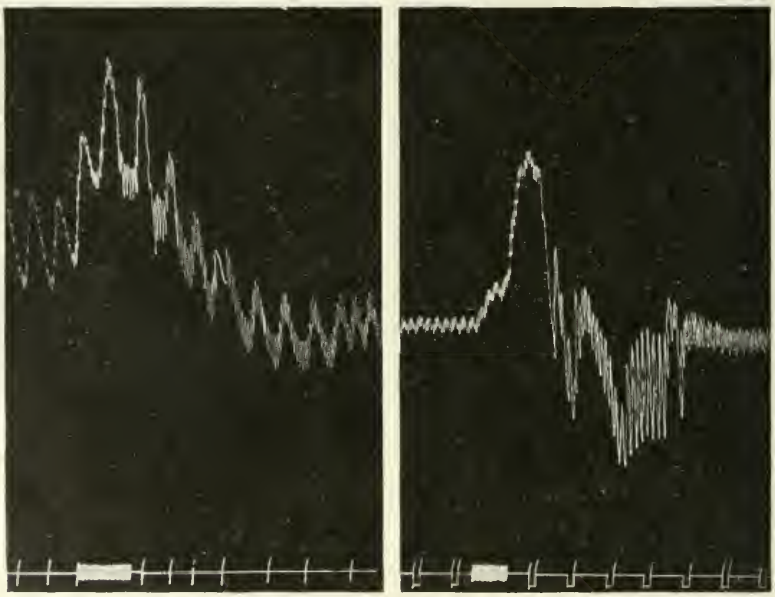
FIGURE 1. Dog 51. (*a*) 1:45 P.M. Reaction to 0.4 c.c. adrenalin, 1:20000. (*b*) 5:58 P.M. Reaction to same quantity adrenalin. Time, 5 sec. Adrenals ligated 2:10 P.M.

first record (*a*) was secured at 1:45 P.M. At 2:10 the adrenals were ligated. The animal recovered promptly from the anaesthetic but showed some evidence of shock. It remained more quiet than a dog which had been merely under either for the same length of time. Ultimately, however, it got up and walked about. At 5:58, about four hours after the first determinations, the reaction to 0.4 cc. of adrenalin was again taken. The blood pressure was found but slightly lower than in the initial case. The reaction to adrenalin was approximately the same. The most notable difference in the two records is in the amplitude of pulse. The heart

beat has become strikingly weaker than before the adrenals were removed.

Figure 2 shows somewhat similar conditions in another animal. The interval between the determinations was $4\frac{1}{2}$ hours. The reaction to adrenalin was slightly increased above normal. The record shows the decrease in respiratory waves that was characteristic of all the experiments.

Figure 3 shows three similar determinations but all made after adrenal ligation. The intervals are approximately $\frac{1}{2}$, $6\frac{1}{2}$ and 9 hours after the glands were tied off. The animal at the time of



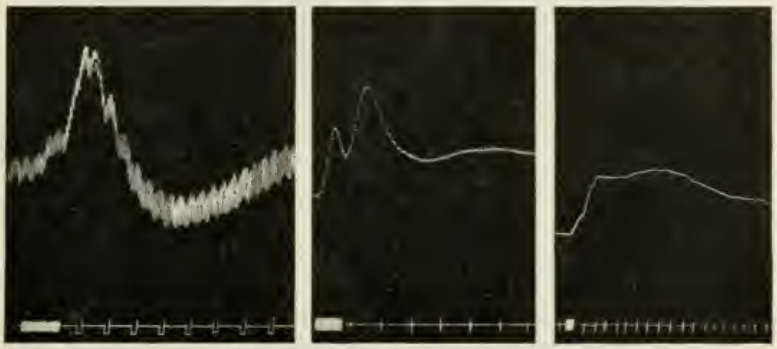
A B

FIGURE 2. Dog 47. (*a*) 11:00 A.M. Reaction to 0.4 c.c. adrenalin, 1:20000. (*b*) 4:30 P.M. Reaction to same quantity adrenalin. Time, 5 sec. Adrenals ligated 10:35. A.M.

the last determination was nearly moribund. It had shown extreme muscular weakness throughout the latter part of the experiment. The records show a persistence of vasomotor tonus and of irritability to adrenalin with the same marked weakening of the heart as in the preceding cases. It is difficult to understand how even with the greatest possible vasomotor efficiency the pressure

of the last determination could have been maintained with a heart too weak to cause an appreciable pulse wave. Figures 1, 2 and 3 show that there is little or no loss of vasomotor tonus or of irritability of the myoneural "receptive substance" even in an animal showing evidence of extreme adrenal deficiency.

Figure 4 shows the reaction of dog No. 50 to 0.8 cc. of 1:2000 nicotin shortly after adrenal ligation and again 4 hours after. It shows the same weakening of heart beat and respiration as in the preceding records and an *augmented irritability to nicotin*. If, as



A B C

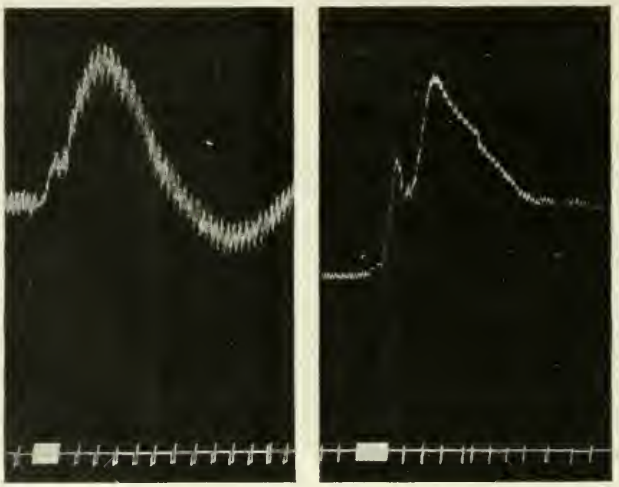
FIGURE 3. Dog 49. (*a*) 10:45 A.M. Reaction to 0.4 c.c. adrenalin, 1:20000. (*b*) 4:58 P.M. Reaction to same quantity adrenalin. (*c*) 7:05 P.M. Reaction to same quantity adrenalin. Time, 5 sec. Adrenals ligated 10:20 A.M.

Langley states,[1] the stimulating effect of nicotin is chiefly upon the sympathetic ganglion cells, the observation would indicate that adrenal extirpation results in an augmented irritability of the peripheral sympathetic system. A similar result was noted in other but not all cases. In one instance it was true to a much greater degree. Even though such augmented irritability were characteristic in all cases it might often be masked by the concomitant cardiac weakness.

Figure 5 shows the reaction to nicotin persisting in a nearly moribund animal, nine hours after ligation of the adrenals. Considering the cardiac weakness the extent of reaction is remarkable. It would indicate that there is certainly no loss of sympathetic irritability involved in the symptom complex of adrenal deficiency.

[1] LANGLEY and DICKASON: Journal of Physiology, 1890, xi, p. 297.

Figure 6 shows the reaction of dog No. 49 to sensory stimulation a half hour and nine hours after adrenal ligation. The stimulus was a Faradic current applied as previously described. The secondary coil was in the same position in each case, a distance of 8 cm. from the primary, — and the primary current was the same, — one dry cell. The observation indicates that the whole vaso-



A B

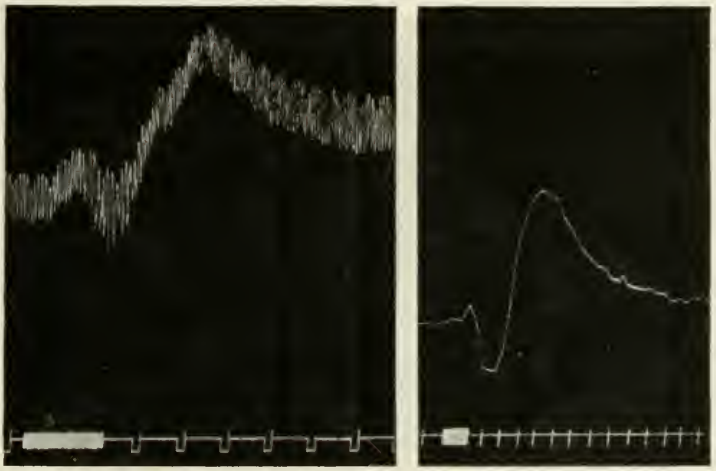
FIGURE 4. Dog 50. (*a*) 11:27 A.M. Reaction to 0.8 c.c. nicotin, 1:2000. (*b*) 3:36 P.M. Reaction to same quantity nicotin. Time, 5 sec. Adrenals ligated 11:05 A.M.

motor arc retains its function at a time when adrenal deficiency has reached an extreme degree.

The results of the series as a whole were consistent and convincing. At a time when the animal showed clearly the characteristic muscular weakness of adrenal deficiency amounting almost to complete paralysis of the hind limbs the vasomotor system was not in the least impaired. As the condition progressed the heart beats became remarkably weak, but a significant lowering of blood pressure did not appear until a late stage. This fact shows that some sort of compensatory reaction must have occurred. That this reaction is an augmented sympathetic irritability is specifically indicated by several nicotin experiments. The lowered blood pressure that finally developed is to be ascribed to primary cardiac failure.

The results obviously show also that the smooth muscle of the arterioles and capillaries does not share in the general myasthenia. This fact which was quite unexpected made the solution of our problem easier than we had hoped.

That shock is not a factor in our experiments is shown by the fact that the results — as in Figures 1 and 3 — were the same



A B

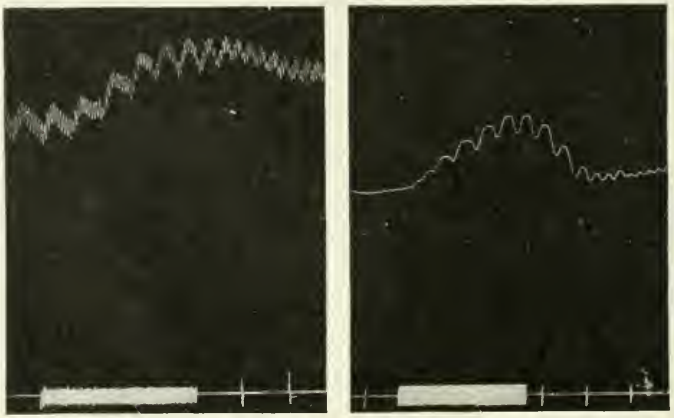
FIGURE 5. Dog 49. (*a*) 10:40 A.M. Reaction to 0.8 c.c. nicotin, 1:2000. (*b*) 7.03 P.M. Reaction to same quantity nicotine. Time, 5 sec. Adrenals ligated 10:20 A.M.

whether the initial determination preceded or followed ligation of the adrenals.

One fact was noted, the significance of which is not clear. In most instances the effects of the nicotin and the adrenalin injections persisted notably longer after ligation of the adrenals than before. Figures 1 and 3 show characteristic instances. Our data do not indicate whether this persistence is to be ascribed to a delayed destruction of the drugs, to a change in the reacting tissues or merely to a slowed circulation whereby the reacting tissues are longer exposed to the stimulating substance. The records as a whole indicate that the effect is not to be ascribed to improved heart action.

In view of the importance that has been ascribed to the adrenal as a "hypertensive" gland the literature shows a surprising dearth

of exact pertinent information. We have found but two accounts of preceding experimental investigations such as that herein reported. Elliott has stated in an abstract report that cats in a moribund condition after adrenal extirpation no longer react to nicotin. Gautrelet and Thomas[1] in 1909 reported in a short "comptes rendus" article the results of another investigation of sympathetic irritability after adrenal extirpation. They reached a conclusion



A B

FIGURE 6. Dog 49. (*a*) 10:50 A.M. Faradic stimulation Crural nerve. (*b*) 5:06 P.M. Stimulation same nerve with same strength current. Adrenals ligated 10:20 A.M. Time, 5 sec.

directly opposed to ours. Their results in brief are as follows: Faradic stimulation of the cervical sympathetic nerve on one side gave a mydriasis with the secondary coil of the inductorium at 10 cm. Five hours after adrenal extirpation a similar stimulation of the cervical trunk on the other side gave a mydriasis only when the secondary coil was at 7 cm. Similarly stimulation of afferent nerves or of the splanchnic trunk when the animal was under the influence of adrenal deficiency failed to affect blood pressure although the splanchnics had been proven irritable during the course of the operations upon the adrenals. They noted also in a rabbit a congestion of the ear which was influenced neither by heat nor

[1] GAUTRELET et THOMAS: Comptes rendus de la Société de Biologie 1909, p. 388.

by cold. They concluded, therefore, that within five hours after adrenal extirpation in both dogs and rabbits the sympathetic nervous system undergoes a loss of irritability. Their results obviously did not show whether the depression was in the nervous elements or in the effector mechanisms. The unfortunate brevity of their report renders a critical consideration of the work impossible. In the light of our experiments it can only be conjectured that their animals as well as Elliott's may have been so nearly moribund as to be incapable of giving any definite information. We have pointed out in a preceding paragraph the desirability of studying the problem in animals that have not yet reached such an extreme stage. It is to be noted that in dogs, as occurred in one of our own experiments, the animal may succumb within six hours of the extirpation.[1]

Schwartz[2] has made certain incidental observations that might be interpreted as bearing upon the problem under discussion. He has found that rats deprived of their adrenals although surviving in apparent good health, within two or three days acquire an augmented sensitiveness to epinephrin. The case of one animal is described particularly: Six weeks after epinephrectomy the animal was given subcutaneously 0.2 mg. of the drug, — a dose which in normal rats is ineffective. It developed extreme restlessness and dyspnea. It ran about the cage frothing at the mouth and with blood coming from the nostrils. Examination after death showed punctiform ecchymoses in all the serous membranes and extreme edema of the lungs, indicating vascular hypertension as the cause of death. The observation so far as it goes accords with our finding of increased sympathetic irritability.

Battelli,[3] in 1902, in a study of adrenal extirpation in which circulatory conditions were particularly under observation found sudden arrest of the heart to be the characteristic cause of death. This finding agrees with ours that cardiac weakness is a primary result of adrenal destruction.

At first thought our results might seem to accord with previous reports postulating a specific relationship between epinephrin and

[1] Gradinescu: Archiv für die gesamte Physiologie, 1913, cli, p. 203.

[2] Schwartz: *Ibid.*, 1910, cxxiv, p. 281.

[3] Battelli: Comptes rendus de la Société de Biologie, 1902, p. 1138.

striated muscle. Researches in Cannon's laboratory[1] have recently shown that a temporary improvement in the reactions of a fatigued muscle undoubtedly follows the injection or discharge of epinephrin. A simple explanation for our observations would be that adrenal extirpation simply reduces the quantity of circulating epinephrin below the minimal amount necessary to maintain muscular metabolism. Several facts, however, oppose such a theory: In the first place if *epinephrin deficiency* were the significant factor it could easily be compensated for by continuous intravenous infusion of the drug. But Battelli has shown that such procedure is absolutely futile. The survival time is either not at all affected or else is actually shortened. Gradinescu[2] in more recent experiments has found that occasional epinephrin injections prolong life somewhat but exert no more than a temporary benefit. The animals invariably die in any case. That there is such a thing as normally circulating epinephrin is pure assumption. That adrenal discharge occurs as a result of various unusual conditions there is no doubt,[3] but there exist no reliable determinations of an epinephrin content of blood collected under normal conditions. Epinephrin in detectable amount does not exist in arterial blood collected from a quiet animal by cardiac puncture.

Moreover, what direct evidence there is indicates that it is the loss of cortical, not chromaffin tissue that leads to the fatal issue of adrenal extirpation. Biedl's experiments upon selachians are well known.[4] Compensatory hypertrophy of adrenal fragments has often led to survival of an animal after extirpation of the glands, but in such cases it is the cortical tissue alone that hypertrophies. Weed[5] has recently reported a crucial experiment upon the point. In an animal which had been preserved by a surviving adrenal fragment ligatures were so placed that the circulation of the cortical part was destroyed, but the chromaffin moiety left unaltered. The animal promptly died. The greater liability to muscular

[1] Cannon and Nice: This journal, 1913, xxxii, p. 44. Gruber, *Ibid.*, 1914, xxxiii, p. 335.

[2] Gradinescu: loc. cit.

[3] Cannon: This journal, 1914, xxxiii, p. 356.

[4] Biedl: Innere Sekretion. Berlin, 1913.

[5] Weed: Verbal communication before the American Society of Experimental Pathologists, Dec. 30, 1913.

fatigue after epinephrectomy observed by Albanese[1] and by Boinet[2] is equally explicable on either hypothesis. Dessy and Grandis'[3] report that application of epinephrin has a long-continued sustaining effect upon frog muscle one of us[4] has been unable to confirm. Moreover, the characteristic syndrome of Addison's disease may develop in patients in whom the cortical tissue alone is affected.[5] On the whole, therefore, it seems most likely that the brief beneficial effect of epinephrin upon striated muscle is but a part of its emergency function and that the characteristic myasthenia following adrenal extirpation is due to cortical deficiency.

Summary and Conclusions

1. Complete ligation of both adrenal glands of dogs at a single operation causes within 4 to 6 hours characteristic weakness of the skeletal muscles, — including those of respiration.

2. The weakness is shared to a marked degree by the cardiac muscle.

3. At a time when cardiac weakness is strongly in evidence blood pressure remains at or near its initial height.

4. A compensatory activity of the vasomotor system therefore occurs.

5. Vasomotor responses to Faradic stimulation of the crural nerve persist. The vasomotor reactions to adrenalin also persist undiminished. The reactions to nicotin are often somewhat exaggerated as compared with preliminary observations with the same dosages.

6. The vasomotor system therefore as well as the vascular musculature are unimpaired at a time when marked asthenia of skeletal and cardiac muscle has developed.

7. This asthenia is sufficient alone to account for the final fatal results of adrenal extirpation.

8. We find no evidence, therefore, that the sympathetic system suffers primarily in any degree from adrenal extirpation.

[1] Albanese: Archives italiennes de Biologie, 1892, xvii, p. 243.

[2] Boinet: Comptes rendus de la Société de Biologie, 1895, xlvii, pp. 273, 498.

[3] Dessy et Grandis: Archives italiennes de Biologie, 1904, xli, p. 223.

[4] Hoskins: Experiments not hitherto reported.

[5] Löwy: Deutsches Archiv für klinische Medizin, 1913, cx, p. 373.

THE RELATION OF PULSATION TO FILTRATION

By ROBERT A. GESELL

[*From the Physiological Laboratory of Washington University*]

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The Effect of Pulsation on Filtration

THE effects of vibration on living protoplasm and on colloids have been pointed out by various investigators and cursorily reviewed in this journal.[1]

Erlanger and Hooker,[2] and later Hooker,[3] pointed out the relation of pulse pressure to renal secretion. More recently I [4] have studied the relation of pulse pressure to renal secretion in the intact kidneys of the dog, by modifying the pulse pressure normally existing in that animal. With the methods employed for changing the pulse pressure no appreciable change in the volume flow of blood through the kidneys was noted. Therefore, the changes in urinary secretion accompanying changes in pulse pressure were ascribed to some specific effect of pulsation itself.

In the experiments cited, pulsation had a marked effect upon the rate of secretion and upon the urea, sodium chloride, and albumin content of the urine. As a rule the rate of secretion and the urea and sodium chloride content of the urine were greater during the periods of pulsatile than during the periods of constant pressure. In a few experiments in which albumin appeared in the urine the amount secreted was greater during the periods of constant than pulsatile pressure.

Whether comparable results might not be produced under more artificial conditions seemed an interesting and important question.

[1] Gesell: This journal, 1913, xxxii, p. 70.

[2] Erlanger and Hooker: Johns Hopkins Hospital Reports, 1904, xii, p. 346.

[3] Hooker: This journal, 1910, xxvii, p. 24.

[4] Gesell: *loc. cit.*

Therefore, various solutions were filtered through different kinds of membranes in the hope that some light might be thrown upon the effect of pulsation upon secretion.

The apparatus employed is shown diagrammatically in figures 1, 2 and 3.

The high pressure filtration apparatus consists of pieces 1, 2, 3,

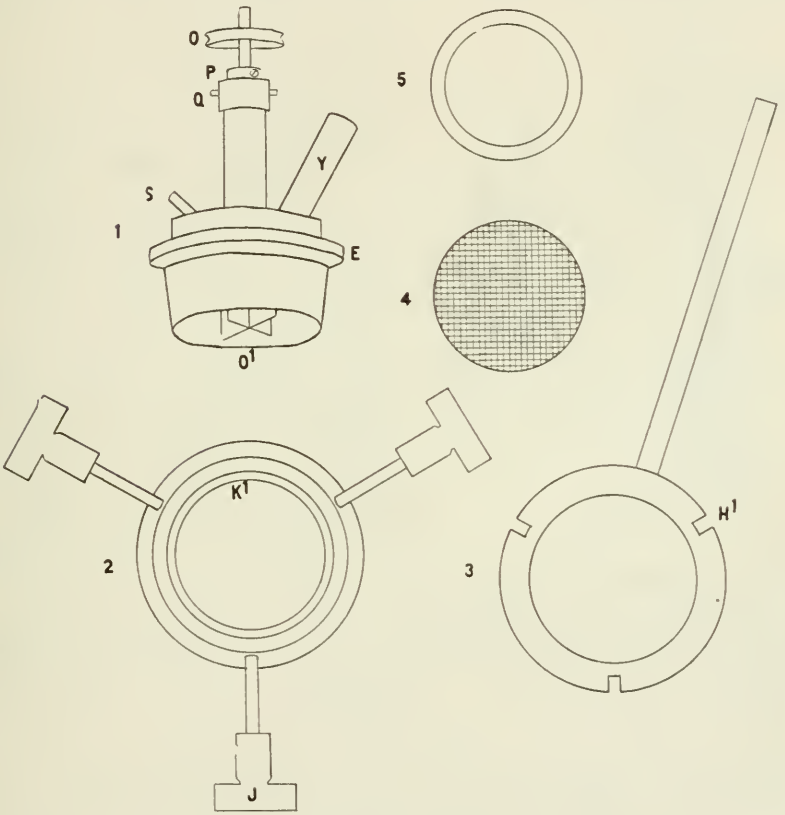

FIGURE 1

4 and 5 in Figure 1. See also Figure 3. The membrane employed is placed on number 5, a thin aluminum ring, and the whole placed upon a supporting perforated disc, number 4. The disc, in turn, is placed in number 2, where it rests securely upon ridge *K'*. Rubber washers are then placed over the membrane. Number 1 fits into number 2. Number 3 fits over ridge *E* of number

1, and adjustable arms *J* of number 2 fit into notches H^1. This arrangement serves to bring the circular edge of number 1 down securely on to the washers over the membrane. *O′* is a four-blade stirring wheel attached to a shaft passing through the packing box, *Q*, and is run at high speed by a motor belted with pulley *O*. *P* is a cuff fitted to the movable shaft; thus the distance between the stirrer and the membrane can be adjusted. *Y* is a large tube through which the pressure upon the membrane is transmitted. *S* is a smaller tube used for filling the apparatus with the filtrans and to record later the pressures prevailing in the filtrans.

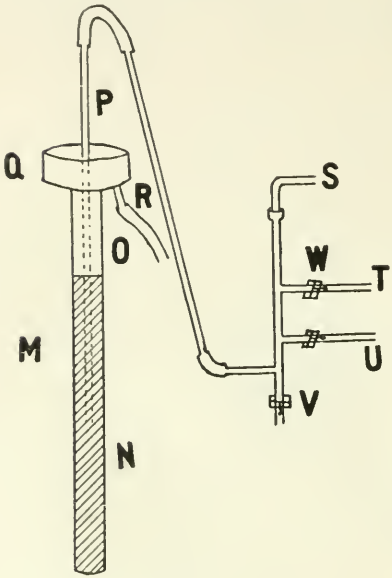

FIGURE 2

Pressures of 15–760 mm. Hg were employed. City water pressure was used as the source of pressure. In order to maintain this at a constant level the arrangement shown in Figure 2 was employed. *N* is a high glass tube containing mercury and *P* a graduated glass tube immersed in the mercury and connected by pressure tubing to hydrant, *S*. Pressure exerted through tubes *T* and *U* was regulated by simply raising or lowering tube *P*. The hydrant was then opened until a fairly brisk stream of water issued from the immersed end of tube *P*. The excess of water required to produce the desired pressure was drained from reservoir, *Q*, through tube *R*.

To convert the constant pressure prevailing in either tube *T* or *U* into a pulsatile pressure, the arrangement shown in Figure 3 was employed. The heavy filtering apparatus was put together as described. A glass cylinder *E* was firmly connected by a rubber stopper to the tube *Y*. The top of the cylinder contained another rubber stopper through which passed a stopcock, *A*, and the right-angle arm of a large calibre *T* tube, *B*. A soft rubber tissue bag containing a couple of drops of Hg was tied tightly about the

right-angle arm protruding into the glass cylinder. The tubes *K* and *F* were connected with the constant head of pressure. Into *K* was fitted a rotating stopcock, by means of which constant pressure was converted into pulsatile pressure. *F* is used to deliver constant pressure. *W* is a Hürthle manometer and *Z* a tube connected with the source of filtrans — both in connection with the filtering chamber through the tube *S*. The chamber was filled

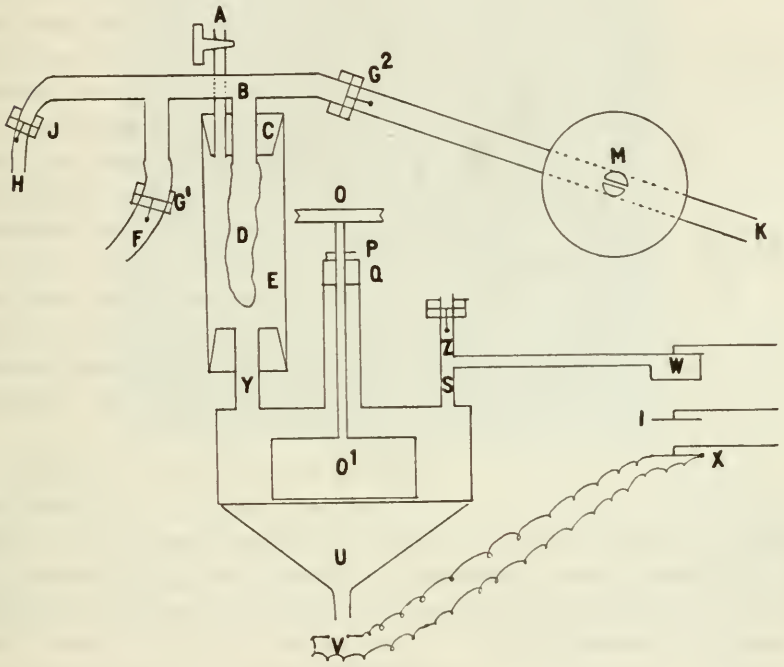

FIGURE 3

with filtrans by opening cocks *A* and *Z*. Cocks *A* and *Z* are closed when the cylinder, *E*, and the manometer system are filled to the exclusion of all air bubbles. The apparatus was then ready for filtration.

If constant pressure is desired clamp G^2 is closed and clamps G^1 and *J* opened and adjusted to produce any desired pressure. With G^1 open, the water rushes into the rubber bag, *D*, and exerts its pressure from the inside of the bag without mixing with the filtrans. As filtration proceeds the space made by lost filtrans is

occupied by water in the bag, and may occupy a large portion of glass chamber, *E*. The filtrans can then be renewed by momentarily closing clamp G^1 and expressing the water in the rubber bag by admitting fresh filtrans through *Z*.

If pulsatile pressure is required clamp G^1 is closed, G^2 opened, and the rotation stopcock, *M*, set in action by motor and pulley. Cock *M* is so constructed that communication between the filtrans and constant head of pressure is made and broken very abruptly so as to effectually produce sudden pressure changes. When cock *M* opens the pressure in *D*, and therefore in *E*, suddenly becomes approximately equal to the source of pressure in *K*. On closure of the stopcock the small amount of water which has accumulated in *D*, due to a certain amount of give in the apparatus, escapes through tube *H* and lowers the pressure in *E*. The clamp, *J*, regulates the amount of fluid escaping from tube *H* and therefore the fall in pressure during the period of closure of the stopcock, *M*. With tube, *H*, wide open systolic pressure can be made to rise to 760 mm. Hg and the diastolic pressure fall to zero. Gradually closing clamp, *J*, — diastolic pressure is rapidly raised, systolic pressure slowly raised, so that the pulse pressure gradually decreases to zero with the clamp entirely closed.

The rate of pulsation is regulated by the speed of the motor. If stirring is required a belt is slipped into pulley *O*. The pressure prevailing in the filtration chamber is recorded by means of the Hürthle manometer, *W*, the rate of filtration by drops, as it falls from the glass funnel *U*, onto the drop recorder *V.X*. Time is recorded in seconds by *I*.

Figure 4 is a record of pulsatile pressure produced by the method described.

In the experiments, alternating periods of constant and pulsatile pressure were used. At the outset, it is of importance to decide what constant pressure should be employed. If filtration is more rapid during pulsatile pressure than during constant mean pressure, pulsation might be considered as having some effect. The criticism might be raised that the greatest amount of filtration occurred only at systolic pressure, and therefore any experiment in which mean pressure was used as constant pressure would not be acceptable. But in the experiments performed the rate of

filtration did not change abruptly when a certain pressure was reached, but roughly varied with the magnitude of the pressure. During pulsatile pressure there is, with every pulsation, a long period of relatively low pressure, and a short period of relatively high pressure. During the long period, filtration is slower than during the short period. The mean pressure which divides these periods

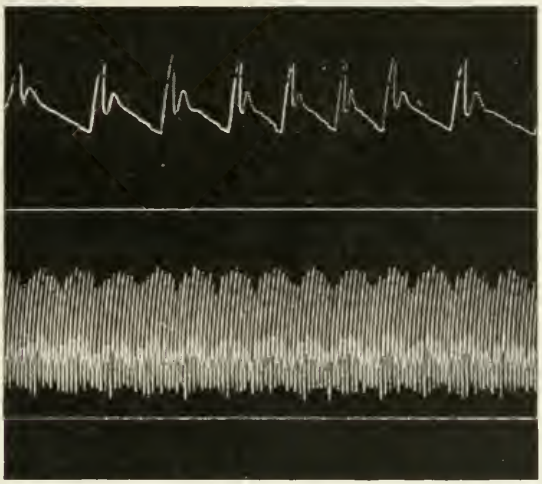

FIGURE 4

should, therefore, give a pressure which acting through the duration of a pulsation should be as effective as the combination of a period of low and high pressure prevailing in the pulsation, — provided pulsation itself has no effect upon filtration.

TABLE I

FILTRANS. — 2% NaCl.
MEMBRANE. — Heavy, coarse, loose mesh paper.
NO STIRRING.

Interval	Pulsatile or constant pressure	Mean pressure in cm. H_2O	No. of drops of filtrate per minute
5	P.P.	135	75
6	C.P.	135	32
7	P.P.	135	38
8	C.P.	135	23

Table I gives the results of an experiment in which a 2 per cent solution of sodium chloride was used as filtrans, and a piece

of heavy coarse loose mesh paper, 1 cm. in diameter, as membrane. Constant and mean pressure of the pulsatile pressure were each 135 cm. of H_2O.

During pulsatile pressure filtration was rapid, but fell to less than half the rate during the following period of constant pressure. On the return of pulsatile pressure filtration increased, not reaching the initial rate, but exceeding that of the preceding period of constant pressure. During the last period of constant pressure there was again a marked falling off of filtration.

An important point to be noted in this table, which also holds for most of the experiments performed, is that as the experiment progresses filtration gradually diminishes in rate — least, however, during the periods of pulsatile pressure. That is, during periods of pulsatile pressure the membrane tends to recover its original permeability.

TABLE II

FILTRANS. — Defibrinated dog's blood 400 c.c.
7% NaCl 1200 c.c.
MEMBRANE. — S.S. Gehärtete F.P. No. 575.
No STIRRING.
PRESSURE. — Mean pressure (80 mm. Hg) throughout experiment.

Interval	Pulsatile or constant pressure	Relative rates of filtration	Per cent decrease in rate of filtration
1	P.P.	16	
2	C.P.	12	25
3	P.P.	10	16
4	C.P.	7	33
5	P.P.	6	14

Table II gives the results obtained on filtering a mixture of defibrinated blood and 0.7 per cent sodium chloride solution through hard filter paper. Constant and mean pressure were each 80 mm. Hg. The results, although not as marked, are comparable to those shown in Table I. Again, there was a gradual diminution in the rate of filtration throughout the experiment. In no case did the rate of filtration during a period of pulsatile pressure exceed that of the preceding period of constant pressure. Yet, if the percentage decrease during each period is calculated, it will be

noted that the greatest decrease occurred during the periods of constant pressure.

TABLE III

FILTRANS. — Defibrinated dog's blood 800 c.c.
Ringer's solution 1200 c.c.
Urea 10 g.
MEMBRANE. — Heavy, coarse, loose mesh paper as in 1.
NO STIRRING.
PRESSURE. — Mean pressure (80 mm. Hg) throughout experiment.

Interval	Pulsatile or constant pressure	No. of drops for each consecutive minute
1	P.P.	1.5, 1.5, 1.5, 1.5, 1.5, 1.5
2	C.P.	0.5, 0.5, 0.0
3	P.P.	2, 2, 2
4	C.P.	0, 0, 0, 0
5	P.P.	2, 2
6	C.P.	0, 0, 0, 0

Table III gives the results of filtering a mixture of Ringer's solution and defibrinated dog's blood through a loose mesh paper membrane. Constant and mean pressure were each 80 mm. Hg. The results in this experiment are the most striking obtained. During pulsatile pressure fairly rapid filtration occurred. During constant pressure little or no filtration occurred.

The question arises what is the cause for such marked changes in rate of filtration under the two conditions given.

At the close of every experiment in which a colloidal suspension was used as filtrans, a slimy, sometimes rather tenacious membrane was seen adhering to the upper surface of the filter. This slimy layer, which thickens as the experiment progresses, presumably adds more and more resistance to the passage of the filtrans through the membrane. Judging from the small amount of give to the apparatus with each pulsation, there should be little stirring of the filtrans at the membrane. Yet it seemed probable that the main effect of pulsatile pressure, so far observed, might be that of preventing the accumulation and concentration of colloids on the surface of the membrane. Therefore a few experiments were performed in which the filtrans was kept in active agitation by means of a rotary stirrer run at high speed. In these experi-

ments the formation of the slimy layer was considerably prevented. It was noted that the rate of filtration did not decrease as rapidly during the progress of the experiment as in the cases in which stirring was not employed.

TABLE IV

FILTRANS. — Egg white 25 c.c.
Ringer's solution 500 c.c.
MEMBRANE. — 1.5% celloidin membrane.
STIRRING.
PRESSURE. — Systolic pressure (40 cm. Hg) used as constant pressure.

Interval	Pulsatile or Constant Pressure	Stirring S or no stirring O	Drops of filtrate per minute
1	P.P.	S	8, 10, 10, 10, 9, 8, 8
2	C.P.	O	8, 5, 5, 6, 4
3	P.P.	S	5, 7, 10, 8, 9
4	P.P.	O	9, 7, 6, 6, 4, 5, 4, 4
5	P.P.	S	5, 6, 7
6	P.P.	O	7, 6, 4

Table IV gives the results of filtering a mixture of egg white and Ringer's solution through a 1.5% collodium membrane prepared according to the method of Bechhold.[1] Periods of constant and pulsatile pressure, with and without stirring, were employed. Systolic pressure of the pulsatile pressure and constant pressure were each 40 cm. Hg. The experiment shows the importance of stirring. With stirring the initial rate of filtration tended to be maintained for some time, while without stirring, even during periods of pulsatile pressure, the rate of filtration rapidly fell off. During period 2 of constant pressure, equal to systolic pressure of pulsatile pressure, filtration was not any faster than during pulsatile pressure, not even during the first minute, presumably before a membrane could have formed on the filter.

Table V gives results of an experiment similar to the preceding one. In this case a 1% membrane was used, and systolic pressure likewise employed as constant pressure. The results again show the beneficial effect of stirring, not alone during periods of constant, but also during periods of pulsatile pressure, although they are probably more marked during the periods of constant pressure.

[1] BECHHOLD: Zeitschrift für Physikalische Chemie, 1907, x, p. 257.

In number 3, during constant systolic pressure, plus stirring, filtration was not any faster than during the preceding period of pulsatile pressure, plus stirring. During period number 3 of constant pressure the rate of filtration gradually diminished, while in the preceding period of pulsatile pressure the rate increased.

TABLE V

FILTRANS. — Egg. white 25 c.c.
Ringer's solution 500 c.c.
MEMBRANE. — 1% glacial acetic collodium membrane.
STIRRING.
PRESSURE. — Systolic pressure (40 cm. Hg) used as constant pressure.

Interval	Pulsatile or constant pressure	Stirring S or no stirring O	Drops of filtrate per minute
1	P.P.	O	10, 11, 9, 8, 8, 7, 8, 6,
2	P.P.	S	7, 10, 11
3	C.P.	S	11, 10, 9, 10, 8, 9, 9, 10, 7, 7, 10, 10, 9
4	C.P.	O	9, 7, 6, 7, 6, 5, 4, 5, 5, 4, 5, 4, 4, 5, 3, 4, 4, 4, 4, 4, 4, 3, 4
5	C.P.	S	5, 6, 7, 7, 7, 7, 8, 7, 8, 6, 7, 5, 6, 7, 7

TABLE VI

FILTRANS. — Ringer's solution 1000 c.c.
Casein 30 g.
Phenolphthalein.
$Ca(OH)_2$ to slightly alkaline reaction.
MEMBRANE. — Dried peritoneum of the dog.
STIRRING.
PRESSURE. — Systolic 75 cm. Hg. Diastolic 70.
Constant pressure 73 cm. Hg.

Interval	Pulsatile or constant pressure	Drops per minute of filtrate
1	P.P.	56
2	C.P.	46
3	P.P.	48
4	C.P.	37
5	P.P.	44
6	C.P.	34
7	P.P.	33

Table VI gives results of filtering through the dried peritoneum of the dog, a mixture of calcium caseinate in Ringer's solution slightly alkaline to phenolphthalein. Alternating periods of constant and pulsatile pressure with constant stirring were employed. A pressure somewhat above mean pressure was used as constant pressure. As in most experiments there was a tendency of the rate of filtration to decrease with the progress of the experiment, but in this experiment that tendency was not very marked. In fact in two instances, periods 3 and 5, of pulsatile pressure the rate of filtration was more rapid than in the respective preceding periods of constant pressure.

Changes in the Nature of the Filtrate

A number of experiments were performed to determine whether there were any qualitative differences in the filtrate passing through the membrane during periods of constant and pulsatile pressure. In all of these experiments the rotary stirrer was run continuously at high speed, and alternating periods of constant and pulsatile pressure used as before. As constant pressures mean and diastolic pressure of the pulsatile pressure were employed.

Berlin blue, calcium caseinate, egg albumin, defibrinated dog's blood and milk, diluted with 0.9% sodium chloride or Ringer's solution, were filtered through collodium membranes and dog's peritoneum.

Such diffusible substances as urea and sodium chloride, with the methods employed for their detection in the filtrate, showed no quantitative relation to constant and pulsatile pressure.

Only in a few experiments did there seem to be a definite relation between the amount of colloids in the filtrate and the type of pressure employed during filtration. When a mixture of Berlin blue and a 1% sodium chloride solution was filtered through a collodium membrane there was no evident relation between the amount of blue in the filtrate and the type of filtration pressure employed.

Casein, in whole milk, diluted with Ringer's solution, filtered through a collodium membrane slightly permeable to casein, also showed no relation between the amount of casein in the filtrate and the type of filtration pressure employed.

The same results were obtained on filtering a 3% solution of calcium caseinate in Ringer's solution slightly alkaline to phenolphthalein, through the dried peritoneum of the dog. The filtrate on standing, however, showed a flocculent precipitation of casein. In the filtrate of pulsatile pressures the precipitation was coarser and occurred more rapidly. Furthermore, the reaction of the filtrans on passing through the membrane was evidently changed — for the filtrate from constant and pulsatile pressure was whiter than the filtrans, but less white from the periods of constant pressure than from the periods of pulsatile pressure. Nothing definite concerning these results can be stated. Further experiments are necessary.

Filtration of egg albumin through collodium membranes showed no quantitative relation to the type of filtration pressure employed. Neither did defibrinated dog's blood, diluted with Ringer's solution, when filtered through collodium membranes show any relation between the amount of albumin filtered and the type of filtration pressure.

In three experiments, however, in which a mixture of defibrinated dog's blood and Ringer's solution was filtered through the dried peritoneum of the dog, with the methods employed for the detection of globulin and albumin, there seemed to be a definite relation between the amount of globulin in the filtrate and the kind of pressure employed. Albumin was tested for and showed no relation to the type of pressure. The tests for globulin gave a cloudier solution with filtrates obtained during periods of constant than during periods of pulsatile pressure. Of these three experiments — diastolic pressure was used as the constant pressure in two, and mean pressure as constant pressure in the other. Even though higher pressures prevailed during the periods of pulsatile pressure, less globulin was forced through the membrane than during periods of relatively lower constant pressure.

Theoretical Considerations

Concerning the *causal* relationship of the changes in the nature of the filtrate accompanying changes from constant to pulsatile pressure, nothing definite can be offered.

The first thing that comes to mind is that either the pores in the membrane are larger during constant pressure than during pulsatile pressure, allowing the passage of colloidal particles through the membrane, or that the colloidal particles are larger during pulsatile pressure than during constant pressure, and therefore cannot pass through the membrane.

The first view does not seem tenable, for, if the pores of the membrane were larger during periods of constant pressure than during periods of pulsatile pressure, the rate of filtration should also be more rapid. This, however, was not the case.

The latter view would seem the more probable explanation: that is, the effect of pulsation may be due to the formation of molecular aggregates, too large to pass the pores of the membrane. Whether this formation of molecular aggregates would most likely occur at the surface membrane (junction between the filtrans and peritoneum) or throughout the filtrans, by agglutination of particles that may be brought into more intimate contact by pulsation, is hard to say.

In this regard the work of Ramsden is significant. Ramsden [1] studied the effect of shaking upon a solution of egg albumin. A clear solution, he found, becomes turbid with the production of fine coagulated strands of albumin which are no longer soluble in the medium. More recently [2] he has extended his experiments to other solutions and suspensions. He found, "that quite apart from evaporation, solid or highly viscous coatings are spontaneously and more or less rapidly formed upon the free surfaces of all proteid solutions; that similar coatings of solid or highly viscous matter occurs on the free surfaces of a large number of non-proteid colloid solutions and fine suspensions, and of a few apparently crystalloid solutions, and that they are formed also at the interfaces of solutions which without being of high viscosity are capable of persistent emulsion."

Ramsden found, "that by simple mechanical means adapted to produce heaping up of surface membranes, large masses of solids (mechanical surface aggregates) can be separated from all pro-

[1] Ramsden: Mann's Chemistry of Proteins, p. 275.

[2] Ramsden: Proceedings of the Royal Society of London, 1904, xxii.

teid solutions and from a large number of colloid solutions and suspensions."

Winkelblech [1] also demonstrated the formation of surface membranes between solutions of gelatin, egg albumin and other solutes and benzene.

Such surface membranes also form at the junction of a solid and a liquid. In the experiments cited in this paper the peritoneum might be considered the solid, and the blood the liquid. At the junction of the peritoneum and the blood there might be formed, by the concentration of colloids, a thick surface membrane. If no "means adapted to produce heaping up of surface membranes" are used, for instance, if constant pressure is employed for filtration, the formation of larger mechanical surface aggregates will not be encouraged. Therefore, if the particles on the membrane are smaller than the pores of the peritoneum, the colloid should be found in the filtrate.

Pulsation, however, is favorable to the formation of molecular aggregates, and, theoretically, should heap up the surface membrane with the formation of molecular aggregates, possibly large enough to prevent their passage through the peritoneum. This explanation of passage of colloids through the peritoneum requires changes in the filtrans only at the surface membrane, namely, the junction of filtrans and peritoneum, and therefore the process must not necessarily be reversible, or at least not quickly reversible.

Another possible explanation is that pulsation might favor the agglutination of the suspended colloidal particles, not only at the surface membrane, but throughout the filtrans. If this be the case, the process must be rather quickly reversible, for the difference in passage of colloids through the peritoneum during constant and pulsatile pressures can be demonstrated repeatedly in the use of one and the same sample of filtrans. So far the apparatus required for the investigation of this point has not been available.

Other less definite suggestions might be offered, but at present the formation of molecular aggregates seems most likely.

[1] Winkelblech: Zeitschrift für angewandte Chemie, 1906, p. 1953.

The Rate of Filtration during Constant and Pulsatile Pressure

In considering the effect of pulsation on the rate of filtration, there are three possible factors to keep in mind: 1. The size of the pores of the membrane; 2. The size of the colloidal particles to be filtered; and 3. The nature of the surface membrane, that is, the junction between the filtrans and filter. The possibly greater effect of a sudden impact in driving smaller particles through a membrane should also be kept in mind.

1. Size of pores. This may be considered from two aspects: (*a*) During pulsatile pressure the high pressure momentarily obtaining at the maximum level might stretch the membrane and so increase the size of the pores. This does not seem very probable for two reasons. One is, that rather thick and strong membranes were used, and they were supported on a perforated plate with circular holes one half mm. in diameter. It seems that a very great force would be necessary to stretch the membranes over such a small orifice. The part of the membrane supported is considerably greater than that unsupported. The compression of the supported membrane, therefore, might more than counteract any stretching over the unsupported areas.

Another thing which speaks against the stretching of the pores by the pressure obtaining during the height of the pulsatile pressure is that no more colloids are found in the filtrate during pulsatile pressure than constant pressure, indeed, in a few experiments, less have been found. It is possible, however, that there may be a balance between the amount of stretching of the pores and the relative increase in size of the molecular aggregates over that of the original suspended colloidal particles.

(*b*) During constant pressure the membrane may be compressed and, therefore, the size of its pores decreased.

Bechhold[1] points out the importance of the elasticity of the membrane for the difference in rate of filtration which he noted on filtration under constant pressure and slow intermittent pressure (not a true pulsatile pressure) in which the pressure was more or less slowly elevated and maintained for 15 minutes, and then again

[1] Bechhold: "Gedenkbock — Van Bemmelen," 1910.

released for the same time. Bechhold suggests that on increase of pressure, the membrane is compressed and the filtrans in the membrane is pressed to the farther side as filtrate. On the release of pressure the membrane due to its elasticity comes back to its normal size and like a sponge sucks itself full of filtrans again. According to Bechhold, intermittent pressure does not destroy the elasticity of the membrane nearly as much as a constant pressure. With the constant pressure the membrane is compressed and the pores necessarily diminished in size.

2. Simple stirring of filtrans. The formation of a rather tenacious membrane on the upper surface of the filter, and its slowing effect on the rate of filtration has been mentioned, also the prevention to a great extent of the formation of this membrane by the continuous use of the rotary stirrer. Stirring had a very marked effect upon filtration during periods of pulsatile as well as of constant pressure. Without stirring, pulsation had a very marked effect upon the rate of filtration and undoubtedly this was due largely to prevention of the accumulation of colloids at the filter. But even with vigorous stirring, pulsation seems to have an accelerating effect upon the rate of filtration. Whether this effect is simply additive to the effect of stirring — that is, produces more effective stirring, is hard to say. If the enhancing effect of pulsation is that of stirring, it is probably stirring of a different nature than that produced by the rotary stirrer, namely stirring by impact.

3. Impact. The velocity imparted to particles of the filtrans at the site of the membrane may be of some importance in helping their passage through the membrane during periods of pulsatile pressure. The fact that larger particles, as the colloids, which have a relatively high inertia, are found in different proportion in filtrates from periods of constant and pulsatile pressure, might support this view.

4. That pulsation might exert an enhancing effect by breaking up of the surface membrane (the junction of the filter and filtrans) which spans the pores or larger irregularities of the membrane is another possibility.

5. Keeping colloids in coarse suspension. Upon the assumption that pulsation favors the formation of molecular aggregates, either

at the filter or throughout the filtrans, the pulsation might favor filtration by keeping the colloids in coarse enough suspension to retard their entrance into the membrane and thereby diminish the clogging effects of these colloids.

Summary

A method is described for filtration with alternating periods of constant and pulsatile pressure.

Numerous solutions were filtered through a variety of membranes under constant and pulsatile pressure — with and without stirring, to determine whether pulsation had any effect: (1) Upon the rate of filtration; (2) upon the nature of the filtrate.

Rate of filtration. In experiments in which the rotary stirrer was not employed, pulsation favored filtration.

Stirring in itself had a very marked enhancing effect upon the rate of filtration, during periods of constant as well as pulsatile pressure.

With continuous stirring, filtration during periods of pulsatile pressure was more rapid than during periods of constant mean pressure.

In a few experiments in which constant stirring was employed, filtration during periods of a constant pressure at the systolic level was not any faster than during periods of pulsatile pressure.

Nature of the filtrate. With the methods for the detection of such diffusible substances as sodium chloride and urea — the amount found in the filtrate bore no relation to constant or pulsatile pressure.

With the methods used for the detection of colloids in the filtrate globulin (dog's defibrinated blood) alone seemed to show a relation to the type of filtration pressure employed — more globulin passing the membrane during periods of constant than during periods of pulsatile pressure.

CONCERNING THE PERIODIC CARDIOVASCULAR AND TEMPERATURE VARIATIONS IN WOMEN

By JESSIE L. KING

[*Department of Physiology, Goucher College, Baltimore, Md.*]

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Historical Introduction.

THE theory that the life-processes in women follow a rhythmical wave-like movement, was suggested first by Dr. Mary Jacobi [1] in 1876. Her observations on the pulse, temperature, blood-pressure and muscular strength have been repeated and extended by a number of other workers and, in general, the conclusions have been that the highest point in all of these processes is reached from two to three days before the onset of the menstrual period, that they sink to the lowest point at its close, gradually rising to normal during the intermenstrual interval.

Much of the literature on this subject has been reviewed by Zuntz,[2] 1906, and by Hansen,[3] 1913, so that, with the exception of the observations on blood-pressure, I shall refer only briefly to it.

Variations in muscular strength during the menstrual period have been studied by Jacobi,[1] Ott,[4] Bossi,[5] Mandl and Bürger.[6] These have, in general, shown a decline corresponding to that of the other life-processes observed.

Although a number of metabolism experiments have been made on pregnant women, von Schroder's [7] furnishes the only reliable study of those menstruating. In three subjects he found a retention of nitrogen immediately before and during the menstrual period. It is an interesting fact that, in dogs, during the proœstrum, Murlin [8] has obtained a corresponding change. Blair Bell [9] records a diminution in the calcium content of the blood. Zuntz [2] states that the minimal exchange of energy is not altered, for the oxygen intake and the carbon dioxide output show no periodic variations.

Changes in temperature during menstruation have been more extensively observed than the changes in any of the other life-processes. Beginning with Retabeau's [10] work in 1870, investigations have followed by Jacobi,[1] Goodman,[11] Stephenson,[12] Reinl,[13] Ott,[4] Giles,[14] Vicarelli,[15] Mandl and Bürger,[6] Riebold,[16] Van de Velde,[17] Zuntz,[2] etc. The most thorough study is that of Hansen [3] in 1913, in which are given temperature curves not only for menstruating women but for normal pregnancy, confinement and lactation. In these a striking similarity is shown between the temperature curves of a man, of a girl before puberty and of a woman after the menopause as well as of women from whom the ovaries have been removed. Mandl and Bürger [6] have reported cases in which the wave form persists, though the removal of the uterus (the ovaries remaining intact) occasioned a cessation of the menses. Van de Velde [17] has observed the wave form in a case of vicarious menstruation. There seems then to be no doubt that there is a premenstrual rise in temperature, a menstrual and postmenstrual fall, followed by a return to normal, the average differences rarely amounting to more than a degree.

For respiration and pulse rate the curves follow those for temperature, but the wave form is less marked — according to Jacobi,[1] Ott,[4] Mandl and Bürger [6] and Zuntz.[2] Zuntz, who counted the pulse under minimum conditions, reports that during menstruation it is one to four beats below the premenstrual rate; the relation between the postmenstrual and the menstrual as well as that between the inter- and premenstrual was variable.

Blood-pressure observations were made by Jacobi,[1] at various intervals, on six normal subjects, through three menstrual periods, using Mohamed's sphygmograph. She does not indicate whether the tracings were taken under similar conditions. Her results show a minimum tension in the radial artery from one to four days after the cessation of the menses and a gradual return to a maximum seven to eight days before the next onset; occasionally, however, not until the first day of the flow. Stephenson,[12] reporting on four cases, found the arterial tension as measured by sphygmographic tracings — somewhat higher six to seven days before the menstrual period than on the day or two preceding. Ott,[4] with Basch's sphygmomanometer, obtained a fall in thirteen out

of fourteen cases; during the flow the pressure was almost constantly below the average, rising to normal again after the cessation. In 1897, Giles[14] studied seven patients admitted to the hospital for "trifling conditions," his records covering in all nine menstrual phases. Using the Dudgeon sphygmograph, he found the blood-pressure highest on the first two days of the period and on the day preceding, lower during the remainder of the epoch, rising again after the cessation. Wiessner[18] reported, in a brief note, observations made with the Riva-Rocci sphygmomanometer. He recorded the lowest pressure at the height of menstruation and a return to normal three to four days after the flow had ceased. The number of cases is not stated. Mosher,[19] using the Mosso instrument, recorded "under uniform conditions" the pressure in nine normal women. In some cases the records have extended over a period of forty-nine days. Most frequently she obtained a fall before the beginning of the menses, "the maximal fall being coincident with the onset," with a gradual return to normal by the time of cessation.

Mandl and Bürger,[6] 1904, studied two normal cases for fifty-one and fifty-three days. They employed the Gartner tonometer and found the average normal pressure to be from 110 to 120, rising to 150 immediately before the beginning of the menses and falling to 90 at the onset. In cases in which the uterus had been removed, they found the usual periodic rise and fall, while if the operation had included the ovaries also, the typical wave form was absent.

The most recent work, reported very briefly by Bogdanovics,[20] 1910, includes observations made with the Riva-Rocci sphygmomanometer, employing the Recklinghausen broad arm band. He obtained both the systolic and diastolic pressures and estimated the pulse pressure on two hundred and fifty persons, including some women who were pregnant and others who were ill. The number of normal women is not stated. In normal women, however, he reported a premenstrual rise of blood-pressure and a gradual decline after the onset of the menstruation. The maximum pressure fluctuated between 95 and 110 mm., the minimum varied from 36 to 52 mm. and the pulse pressure averaged 58 mm.

Observations and Discussion

My own observations, which I desire to report in this paper, were begun about two and a half years ago, in order to determine whether the periodic rhythm in blood-pressure is altered if regular exercise is taken during the menstrual phase as well as throughout the other phases of the cycle. It was necessary to secure daily records for each individual in order to be assured of the periodic

TABLE I

General Data Regarding Subjects

Subject	Age	Weight kilos	Height cm.	Days of record	Menstrual periods	Average duration of period
Group I						
A	18	59.1	162.5	96	4	3 days
B	17	59.5	165.0	84	3	3 "
C	18	54.88	162.5	93	3	5 "
Group II						
A	18	51.81	162.5	61	2	7 "
B	19	59.1	162.0	51	1	5 "
C	42	56.4	170.0	73	3	3 "
Group III						
A	36	52.27	160.0	79	3	3 "
B	40	63.6	170.0	37	2	3 "
C	32	48.6	166.7	(*a*) 63 (*b*) 57	3 2	4 "

rhythm. I was greatly surprised at being unable to discover, in the four subjects selected, the typical wave form in the blood-pressure curves so generally accepted. Thinking that possibly individual peculiarities were responsible for these negative results, the observations were continued on two other groups under different conditions but with practically the same results.

The subjects selected for this investigation were seven college students and four older women engaged in regular academic work. The ages ranged from 17 to 42 years. (Table I). All were in

normal health and all were in the habit of continuing their regular college duties during the menstrual phase. The college students frequently did not even interrupt their regular gymnasium exercise requirement of three hours weekly.

On the basis of the conditions under which observations were made, I shall separate the individuals into three groups. The

TABLE II

AVERAGES OF GROUP I

Phase	Systolic pressure		Diastolic pressure		Pulse pressure	
(*A*)	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Intermenst.	111.54	108.9	75.42	75.65	37.9	36.9
Premenst.	109.2	111.69	73.42	74.5	35.33	35.19
Menstrual	111.34	111.4	74.2	76.72	36.16	34.0
Postmenst.	110.2	111.8	75.5	77.85	36.0	34.5
(*B*)						
Intermenst.	113.6	110.1	75.0	73.0	36.7	31.7
Premenst.	109.4	111.0	74.57	74.0	34.6	35.0
Menstrual	111.8	111.7	76.2	76.85	35.1	34.8
Postmenst.	113.3	113.3	72.66	75.6	39.0	36.6
(*C*)						
Intermenst.	111.2	114.0	71.97	77.2	37.4	35.8
Premenst.	109.8	115.8	72.0	73.5	36.0	41.2
Menstrual	113.4	114.0	74.6	77.5	45.5	36.5
Postmenst.	110.9	113.0	70.4	76.9	38.0	36.4

Erlanger sphygmomanometer with the standard cuff was employed and graphic records taken of both the systolic and diastolic pressures on the members of groups I and II. The systolic record was always checked by the auscultatory method, and whenever there was any uncertainty regarding the graphic record, that obtained by auscultation has been used.

Group I included four young women, who came to my office in the morning between 8 and 8:45 — half an hour to an hour

after breakfast, and in the afternoon between four and six. In the morning the building was reasonably quiet, in the afternoon there was, at times, a good deal of noise because of the proximity of the gymnasium. Every effort was made to secure identical conditions of quiet and repose in the subjects before observations were attempted. Since the records were continued

TABLE III

AVERAGES OF GROUP II

Phase	Systolic pressure		Diastolic Pressure		Pulse pressure		Temperature	
(A)	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Intermenst.	112.6	114.0	76.15	81.6	34.8	32.4	97.7	98.32
Premenst.	112.75	114.85	73.5	79.7	37.75	34.6	98.41	98.02
Menstrual	112.0	117.4	78.6	82.4	33.2	33.0	98.26	98.5
Postmenst.	112.5	113.0	76.4	80.3	34.5	33.3	98.18	98.4
(B)								
Intermenst.	107.3	113.4	77.7	82.3	28.5	31.4	98.16	98.94
Premenst.	109.1	112.6	79.2	86.75	27.0	30.5	98.3	98.94
Menstrual	108.8	118.4	80.8	86.0	27.2	32.4	98.58	99.6
Postmenst.	106.0	110.0	78.0	81.0	27.5	29.0	98.3	98.6
(C)								
Intermenst.	99.35		72.85		28.0		98.1	
Premenst.	102.75		73.55		28.66		98.1	
Menstrual.	102.57		74.3		28.3		98.05	
Postmenst.	98.0		72.3		425.		98.1	

over long periods, and since the girls were familiar with the surrounding conditions and were accustomed to the noises, it was not felt that they were disturbing factors.

In order to have more constant conditions, however, the observations were undertaken on group II in a room of the infirmary located on the fifth floor of one of the college dormitories. This room was reached by an elevator, thus obviating disturbance due

to exercise. The subjects were four residents of this hall. The morning hour was from 8:15 to 9 and the evening 7:45 to 8:15.

In order to eliminate the possible interference of meals and to have conditions as nearly as possible at a minimum, the blood-pressure determinations were made on a third group of three before they arose in the morning, between six and seven o'clock.

TABLE IV

AVERAGES OF GROUP III

Phase	Systolic	Diastolic	Pulsepressure	Pulse rate	Temperature
(*A*) Intermenst.	96.02	64.27	28.15	63.23	
Premenst.	101.0	67.11	35.0	62.11	
Menstrual	95.27	65.65	29.63	59.63	
Postmenst.	95.8	68.2	27.6	59.36	
(*B*) Intermenst.	115.22	86.22	34.44	55.0	
Premenst.	112.62	80.75	31.87	57.7	
Menstrual	118.66	87.71	30.5	58.0	
Postmenst.	116.6	84.5	32.33	53.0	
(*C*) (*a*) Intermenst.	97.03	78.0	19.6	71.17	99.0
Premenst.	96.8	76.0	20.8	76.2	98.67
Menstrual	98.3	78.1	20.0	74.4	98.69
Postmenst.	101.0	79.3	21.66	75.8	98.9
(*C*) (*b*) Intermenst.	114.0	90.9	23.35	72.4	98.89
Premenst.	111.75	87.0	22.25	72.25	99.32
Menstrual	114.7	89.75	24.25	73.34	98.71
Postmenst.	114.5	91.14	23.6	70.7	98.29

Group III, *C* (*a*) averages of blood-pressure observations made with the Tycos sphygmomanometer, subject in reclining posture, between 6 and 7 A.M.

C (*b*) averages of blood-pressure observations made with the Erlanger sphygmomanometer, subject in sitting posture. Pulse rate and temperature taken with subject reclining, a few minutes before blood-pressures. Hour as in (*a*). Forty-nine days intervened between *a* and *b*.

The Tycos sphygmomanometer and the auscultatory method were used for both pressures. I checked this instrument frequently by a mercury manometer and found it constant and accurate. A

FIGURE I. Curves plotted from daily observations of pulse rate and blood-pressure made on *A* of group III, before she arose in the morning. The auscultation method with the Tycos sphygmomanometer was employed for the blood-pressure determinations. The menstrual periods are represented by the cross-hatched lines. See discussion on page 10, tables IV and VIII.

few weeks after these observations were discontinued, a second series was made under similar conditions on *A* and *C* of this group. On *A*, who was reclining, the Tycos was again used, but on *C*, who was sitting, the Erlanger instrument was employed.

Temperature records were obtained for groups I and II, taken by mouth, and for *C* of group III by rectum. The pulse was counted for one minute in groups I and III and in addition records of rectal temperature and of pulse rate were obtained, under minimum conditions, from two members of group I and for one of group III, the observations extending over periods of from 70 to 96 days.

The following results may be noted:

1. The temperature curves of groups I and II and the pulse curves of group I tend to follow the periodic rhythm indicated by numerous workers. This, however, is more obvious in those cases in which records were taken under minimum conditions (tables IV and Vb, chart II). The rectal temperature is, without doubt, more accurate than that taken by mouth. On one subject observations were likewise made on the rate of respiration and although these cover only forty days, in this case at least, there is an apparent rhythm.

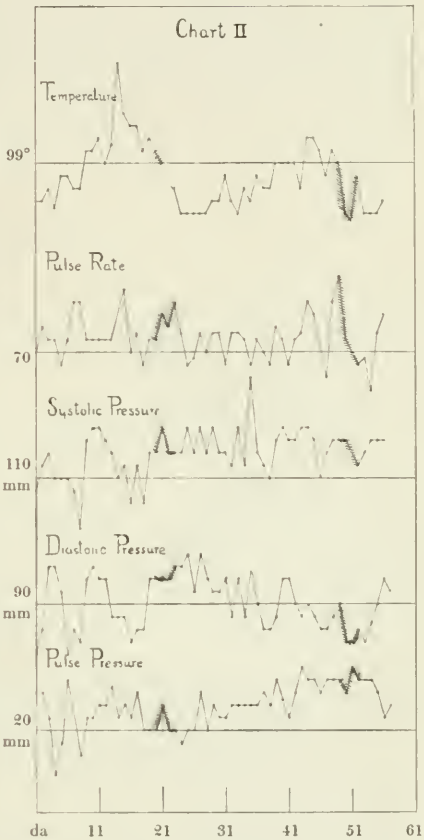

FIGURE 2. Curves plotted from daily observations of rectal temperature, pulse rate and blood-pressure made on *C* of group III. Temperature and pulse taken before the subject arose in the morning, blood-pressure determinations immediately afterward but with subject in sitting posture. Erlanger instrument employed. The menstrual periods are represented by the cross-hatched lines. See discussion on page 10, tables IV and VIII.

2. The blood-pressure determinations in the eleven cases studied have varied in duration from thirty-nine to ninety-six days. They have covered in all twenty-five complete menstrual epochs and thirty periods. The numbers given in the charts were obtained by averaging all

the records of the respective phases for each individual, thus securing a more accurate picture of the real values. The length of the pre- and postmenstrual phases, as I have taken them, depend upon the duration of the menstrual periods. That is, I have made the three periods equal and then considered the remaining days of the cycle as belonging to the intermenstrual interval. The data for two cases, I *D* and II *D*, are omitted since the former presented a marked case of amenorrhea and

FIGURE 3. Curves plotted from daily observations of blood-pressure made on *A* of group I, between 4 and 6 P.M., subject in sitting posture. Erlanger instrument employed. The menstrual periods are represented by the cross-hatched lines. See discussion on page 10, tables II, V, and VI.

will be discussed in a later paper along with similar cases of irregular recurrences. In the record of II *D* there are a number of breaks so that it can hardly be considered a fair case. It should, however, be mentioned that neither gave any indication of a periodic rhythm.

With one exception the blood-pressure curves of all subjects fail to show a rhythmical movement. In fact as great variations may be observed during the intermenstrual phase as between the premenstrual and the menstrual. I feel confident that if the menstrual periods were not indicated on the charts (prepared from the records of each subject), one could not locate them with any degree of certainty.

In the exceptional case, *A* of group III (chart I, table IV) the systolic and pulse pressures undoubtedly show a rise two to three days before the onset of the period, then a gradual decline which continues throughout the menstrual and postmenstrual epochs. I desire to call special attention to the fact that the pressures at these times are not lower, sometimes not as low as those observed

TABLE V

GROUP I, C

(a) *Averages of mouth temperature and pulse rate taken at the same time as the blood-pressure records of chart I*

Phase	Temperature	Pulse rate	Menst. record above or below, Temperature	Pulse rate
Intermenst.	98.65	74.1	− 0.14	− 1.8
Premenst.	98.7	75.28	− 0.19	− 2.98
Menstrual	98.51	72.3		
Postmenst.	98.63	70.93	− 0.12	1.37

(b) *Averages of rectal temperature and of pulse rate taken between 6 and 7* A.M., *before rising 18 mo. later*

Phase	Temperature	Pulse rate	Menstrual records above or below Temperature	Pulse
Intermenst.	98.1	69.9	0.15	− 0 6
Premenst.	98.58	70.5	− 0.33	− 1.2
Menstrual	98.25	69.3		
Postmenst.	97.92	66.75	− 0.33	2.55

here and there during the intermenstrual days. The diastolic pressures show irregularities too great to permit an analysis of the curve. The two exceptional pressures, for reasons indicated on the chart, have been omitted from the averages. I am unable to account for the other striking variations.

C of group III (chart II, table IV) shows, undoubtedly, a periodic rhythm in the temperature variations and possibly in the pulse rate, but no such periodicity can be detected in the blood-pressure curves.

In *A* of group I the objection may be raised that conditions were not sufficiently constant to give the normal record in blood-pressure, but on comparing pulse and temperature records taken at this time (table V) with others taken in the morning before

TABLE VI

GROUP I. MENSTRUAL RECORDS ABOVE OF BELOW THOSE OF OTHER PHASES

Phase	Systolic pressure		Diastolic pressure		Pulse pressure	
(*A*)	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Intermenst.	− 0.2	2.5	− 1.22	1.1	− 1.7	− 2.9
Premenst.	2.14	− 0.3	0.8	2.2	0.83	− 1.2
Postmenst.	1.14	− 0.4	− 1.3	− 1.13	0.2	− 0.5
(*B*) Intermenst.	− 1.8	1.6	1.2	3.85	− 1.6	− 3.2
Premenst.	2.4	0.7	1.6	2.85	0.54	− 0.15
Postmenst.	− 1.5	− 1.6	3.54	1.25	− 3.9	− 1.7
(*C*) Intermenst.	2.25	0	2.6	0.4	8.13	0.75
Premenst.	3.61	− 1.8	2.57	4.0	9.5	− 4.7
Postmenst.	2.54	1.0	4 17	0.6	7.5	0.1

arising, it may be seen that there are but slight variations in the differences in the two sets of records. Such a comparison would lead one to the conclusion that here the outward conditions were not important modifying factors. It was found more satisfactory to chart the morning and afternoon records separately, in the cases in which both were taken, as well as to prepare the two sets of averages. The blood-pressure curves inserted (chart III) were prepared from the afternoon determinations but are not significantly different from the morning curves.

Reviewing the entire series of observations, including those already discussed, it is evident that there are menstrual epochs in which all three pressures fell below the intermenstrual pressure, but the average differences are slight and on the other hand there are epochs in which they rose above the normal. If the averages for each phase be examined (tables II, III, IV) it will be seen that

TABLE VII

GROUP II. MENSTRUAL RECORDS ABOVE OR BELOW THOSE OF OTHER PHASES

Phase	Systolic pressure		Diastolic pressure		Pulse pressure		Temperature	
(*A*)	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M
Intermenst.	− 0.57	3.4	2.4	0.8	− 1.54	− 0.64	0.56	0.18
Premenst.	− 0.75	2.55	5.1	2.7	− 4.5	− 1.57	− 0.15	0.48
Postmenst.	− 0.5	4.4	2.13	2.1	− 1.3	− 0.33	0.1	0.1
(*B*)								
Intermenst.	1.5	5.0	3.1	3.66	− 1.3	1.0	0.42	0.66
Premenst.	− 0.3	5.8	0.6	− 0.75	0.2	1.9	0.28	0.66
Postmenst.	2.8	8.4	2.8	5.0	− 0.3	3.4	0.28	1.0
(*C*)								
Intermenst.	3.2		1.43		0.3		− 0.05	
Premenst.	− 0.18		0.73		− 0.4		− 0.05	
Postmenst.	4.57		2.0		2.9		− 0.05	

there is a tendency toward a premenstrual rise and a postmenstrual fall, but these differences are not great (tables VI, VII, VIII). The premenstrual rise occurred in the systolic pressures of six individuals, the greatest increase amounting to 5.73 mm. (chart I, table VIII.) The diastolic premenstrual pressure exceeded that of the menstrual in but two cases and in these less than 2 mm. (table VII, VIII). Since the pulse pressure is recognized as being of especial value as an indication of the efficiency of the heart, one might expect to find a regular variation here, yet the highest average difference in eight individuals was but 5.37 mm. (table VIII). The postmenstrual averages in about half of the subjects were a little below the menstrual and in the majority of

cases 1 to 2 mm. lower than the intermenstrual. If the product of the pulse pressure by the pulse rate be considered, it is found to be remarkably constant for each individual.

It is difficult to compare my blood-pressure findings with those of other workers, since they have not tabulated their results and only two have plotted them as curves (Stephenson and Mandl

TABLE VIII

GROUP III. MENSTRUAL RECORDS ABOVE OR BELOW THOSE OF OTHER PHASES

Phase	Systolic	Diastolic	Pulsepressure	Pulse rate	Temperature (rectal)
(*A*)	A.M.	A.M.	A.M.	A.M.	A.M.
Intermenst.	− 0.75	1.38	1.48	− 3.6	
Premenst.	− 5.73	− 1.56	− 5.37	− 3.48	
Postmenst.	− 0.53	− 2.55	2.03	0.3	
(*B*) Intermenst.	3.44	1.5	− 3.94	3.0	
Premenst.	6.04	6.96	− 1.4	0.3	
Postmenst.	2.0	3.2	− 1.8	5.0	
(*C*) (*a*) Intermenst.	1.3	0.1	0.4	3.23	− 0.31
Premenst.	1.5	2.1	− 0.8	− 1.8	0.02
Postmenst.	− 2.7	− 1.2	− 1.66	− 1.4	− 0.21
(*C*) (*b*) Intermenst.	0.7	− 1.15	0.9	0.94	− 0.18
Premenst.	2.95	2.75	2.0	1.1	− 0.61
Postmenst.	0.2	− 1.39	0.65	2 64	0.43

and Bürger). Faught[21] has brought out the difficulty of making comparisons between the figures obtained with the instruments used some years ago and those now in use, especially when the width of the cuff is not stated, or when it is not known whether any cuff was used.

I am aware that there are many possibilities of error in this work and that conclusions cannot be drawn from observations on

eleven subjects. Yet it is of great interest that records taken over long periods, with the most approved of modern sphygmomanometers, give little support to the results hitherto generally accepted.

Various theories have been brought forward to explain the diminution in temperature which undoubtedly occurs. Zuntz [2] concludes that, since his experiments indicate no change in heat production, there must be an increase in the heat given out. He suggests that this may be due to a change in the innervation of the blood-vessels; also that the profuse sweating, which he says occurs in many women during menstruation, may play a rôle in the temperature reduction. The results of my blood-pressure observations do not give much support to the innervation hypothesis, while in regard to the profuse sweating, such a condition did not occur in any of my subjects and seems to be a phenomenon unknown or unnoticed by normal women. According to Hansen,[3] the reduction may be explained on the basis of the decrease in protein metabolism. Dr. F. G. Benedict, of the nutrition laboratory (in a letter to the writer), regards a constantly lower pulse, when taken under minimum conditions, as a clearer proof of lower heat production than a mere falling temperature. One might regard the diminished pulse rate, where it occurs, as of greater significance than the fall in temperature.

Granted that lowering in pulse rate, temperature, and blood-pressure occur during the menstrual and postmenstrual periods, should much significance be attached to a diminution in pulse rate of from two to three beats, to temperature variations rarely greater than one degree, and to blood-pressure changes averaging from two to five millimeters? Certainly one would suppose that in a healthy individual a normal periodic function should not be accompanied by a marked depression in all of the life-processes and a generally lowered efficiency so emphasized by numerous writers. If there is a compensation for the loss of blood by the retention of nitrogen (Murlin),[8] then it would seem that there should be a compensation in the cardiovascular system and this is indicated in the tendency of the blood-pressure to show such slight variations.

Summary

In the study reported, observations made on the pulse and temperature of women support the usually accepted theory of a rhythmical movement in the life-processes; the highest point is reached from three to four days before the menses, the lowest point about three days after their cessation. With one exception, blood-pressure records of the systolic, diastolic and pulse pressures made on eleven women gave such irregular results that they cannot be regarded as supporting the wave-theory.

My results, as far as they go, seem to indicate that there has been a tendency to overemphasize the inefficiency of women during the menstrual period.

It is a pleasure to express my appreciation of the encouragement and helpful suggestions received, during the progress of this work, from Dr. Lilian Welsh and from Dr. Donald R. Hooker. I wish also to thank the latter for the use of one of the Erlanger sphygmomanometers from his laboratory.

I am greatly indebted to my students and to the other women, subjects of the observations, for their intelligent interest and hearty coöperation in the work. One of the group, Miss Marion Janney, has prepared the charts.

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THE INFLUENCE OF CURARE ON VASOMOTOR REFLEX THRESHOLDS

By E. G. MARTIN and P. G. STILES

[*From the Laboratory of Physiology in the Harvard Medical School*]

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IN a recent communication from this laboratory dealing with the thresholds for certain vasomotor reflexes,[1] the point was made that in the ordinary experimental use of faradic stimuli shocks of undue intensity are frequently employed, particularly with curarized animals, in which obvious signs of excessive stimulation are not afforded. In connection with a discussion of the extent to which the use of very strong stimuli is justified the question was raised (p. 225) as to whether curare might not have so depressing an influence on vasomotor activity as to require powerful stimulation to overcome it. The present paper is a report of the results of our studies of this point.

The admirable investigations of Sollmann and Pilcher [2] have shown that *qualitatively* the vasomotor mechanism is not seriously modified by ordinary doses of curare, except for a transient peripheral block. These authors did not consider the question of a possible *quantitative* effect of the drug, since they employed maximal stimuli throughout their work.

Method. — Our experiments were performed upon cats, narcotized with ether. In these experiments we used continuous etherization, carried on thus: two ordinary lamp wicks were inserted in a small bottle of ether with about 5 cm. of each projecting. This bottle was then placed within a larger, wide-mouthed bottle provided with inlet and outlet tubes as in the ordinary ether bottle. By adjusting the exposure of wick a rate of ether evaporation just sufficient to maintain the desired degree of anaesthetization was readily obtained. With this method we had very uniform results.

[1] Martin and Lacey: This journal, 1914, xxxiii, p. 212.

[2] Sollman and Pilcher: This journal, 1910, xxvi, p. 233.

We administered curare by injection into the femoral vein. Our etherized cats were quite resistant to the drug. Thirty mg. per kilo body weight were regularly used, and often did not induce complete irresponsiveness to strong peripheral sciatic stimulation, although spontaneous breathing was abolished. For artificial respiration we used an air blast interrupted by a motor-driven valve.

We observed, as did Sollmann and Pilcher,[1] a marked and immediate fall of blood pressure following the injection of curare. Perhaps on account of our heavier doses, or because we used cats instead of dogs, the blood pressure was slower in returning to a persistent level than in their experiments. We also noted a difference from their results in that the persistent level after curare was lower than the original level, whereas in their animals it was usually higher.

We investigated the effect of curare upon the thresholds for blood-pressure drop and blood-pressure rise from central stimulation of the sciatic and saphenous nerves in the hind leg and of various branches of the brachial nerve in the front leg. We studied also the effect of the same drug on the thresholds for mild depression and profound depression from central stimulation of the combined vago-depressor trunk.[2]

Thresholds were measured in *Z* units.[3] The rate of stimulation varied between 8 and 15 per second.

Results. — Table I contains a summary of our results with sensory nerves other than the vagus. This table indicates a moderate lowering of the sensitiveness of the vasoconstrictor centre by curare, but not in our opinion a sufficient lowering either to invalidate the use of curare in vasomotor experiments, nor to justify the use of stimuli of supraphysiological intensity.

The experiments on central stimulation of the vago-depressor resulted as follows: the threshold for mild depression without curare (9 experiments) averaged 10 *Z* units; with curare (8 ex-

[1] Sollman and Pilcher: *loc. cit.*, p. 239.

[2] Martin and Stiles: This journal, 1914, xxxiii, p. xxxvi; and xxxiv, p. 106.

[3] Martin: The measurement of induction shocks, New York, 1912, p. 73. Also Martin and Lacey: *loc. cit.*

periments) 13 *Z* units. The threshold for profound depression (more than 24 per cent) without curare averaged for 10 experiments 245 *Z* units; with curare (9 experiments) 240 *Z* units. These results, even more clearly than those with other sensory nerves, show how slight is the influence of curare on the vasomotor centres. Although there appears to be no change of the threshold for profound depression by curare, certain facts that have come out in connection with this study suggest that the drug may have a definite effect on the vasoconstrictor centre.

TABLE I

The Influence of Curare on the Thresholds for Reflex Blood-pressure Change. Z Units

	Thresholds for pressure drop				Thresholds for pressure rise			
Nerve Stimulated	No. expts. averaged	Threshold before curare	No. expts. averaged	Threshold after curare	No. expts. averaged	Threshold before curare	No. expts. averaged	Threshold after curare
Sciatic	7	14 *Z*	4	27 *Z*	5	168 *Z*	6	256 *Z*
Saphenous	5	5	4	16	2 [1]	265	3 [1]	500
Branch of Brachial	5	7	5	28	4	400	6	480

[1] We have on five occasions with and without curare failed to obtain a rise in blood pressure from central stimulation of the saphenous nerve with shocks exceeding 2000 *Z* units. Well-marked pressure drops were brought about in these cases by all the stimulations we administered.

In our paper on the two types of reflex blood pressure drop [1] the "all or none" character of the mild depression and the rather abrupt change to the profound type were mentioned. In comparing our series of graded stimulations with and without curare we find that in the curarized animals the change from the mild to the profound drop was apparently less abrupt than in the cases where no curare was used. In other words, the extent of pressure drop appears to bear a closer relation to the stimulation strength in curarized animals than in those that are uncurarized.

In the paper cited above we have described characteristic

[1] Martin and Stiles: *loc. cit.*, p. 110

qualitative differences between the mild and profound types of pressure-drop.[1] These were first noted by Bayliss.[2]

Quantitatively we find that the mild type in uncurarized animals is rarely associated with an extent of drop exceeding 14 or 15 per cent, and that the profound type is rarely less than 20 to 24 per cent. In twelve experiments without curare including 97 mild depressions and 25 profound ones, there were only seven whose extent was between 14 and 20 per cent, whereas in nine experiments with curare, including 59 mild depressions and 25 profound ones, there were 13 with extent between 14 and 20 per cent.

TABLE II

EXPERIMENT OF NOVEMBER 25, 1913

THE GRADATION OF REFLEX BLOOD-PRESSURE DROP IN CURARIZED ANIMALS CONTRASTED WITH THE ABRUPT CHANGE OF CHARACTER FROM MILD TO PROFOUND PRESSURE DROP IN UNCURARIZED ANIMALS FROM CENTRAL STIMULATION OF THE VAGUS NERVE

Without curare		With curare	
Stimulus *Z* units	Percentile pressure drop	Stimulus *Z* units	Percentile pressure drop
1.87	7.1	1.87	10.2
3.25	9.2	22	16.8
5	10.3	45.5	18.9
22	10.8	60	25.7
35	12.3	73.2	32
60	9.2	127	38.5
127	12.3		
192	31		

This tendency toward an increased gradation of response after curarization can be illustrated also by the data from a particular experiment. Table II presents the record obtained on November 25, 1913. The table shows that in the uncurarized animal after

[1] MARTIN and STILES: *loc. cit.*, p. 110..

[2] BAYLISS: Journal of physiology, 1893, xiv, p. 314.

the full, mild effect was reached at 5 *Z* units a 25-fold multiplication of the stimulus to 127 units brought about no noteworthy increase in the extent of response, and that after that point was passed a 50 per cent increase in stimulation strength caused a response two and one-half times as great as the last one, this marking the region of transition from the mild to the profound type of depression. After the administration of curare, on the other hand, the response shows a definite tendency to increase as the strength of stimulus increases. A possible explanation of this effect of curare may be that it impairs the coherence of the vasoconstrictor centre, causing it to respond to sensory stimulation more or less piecemeal instead of as a unit. In the particular experiment cited in Table II there appeared likewise a definite increase of sensitiveness after curare. Our experience as a whole does not suggest that this latter is a necessary or characteristic effect.

Conclusion. — Our results may be summarized in the statements (1) that the thresholds for vasomotor reflexes are not, as a rule, markedly affected by curare; and (2) that the vasoconstrictor centre shows signs of impairment of unity under the influence of the drug, causing it under certain circumstances to exhibit gradations of response not usual in uncurarized animals.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD[1]

I. The Graphic Method of Recording Coagulation Used in These Experiments

By W. B. CANNON and W. L. MENDENHALL

[*From the Laboratory of Physiology in the Harvard Medical School*]

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MANY methods have been devised for determining coagulation time. The description of these methods is unnecessary in this paper, for they have been considered critically in two comparatively recent reviews, one by Addis,[2] the other by Morawitz.[3] With different methods the coagulation time of blood (at 20°) has been set down as ranging from approximately 5 minutes (Addis) to 20 minutes (Morawitz and Bierich). This great discrepancy shows that there is no definite "coagulation time" quite independent of the method used, i.e., the conditions peculiar to any coagulometer are likely to affect the time of clotting. Since to drawn blood any instrument is a foreign body, all that is required of an instrument is that the conditions of its use shall be constant.

The conditions defined by Addis as being essential for accurate estimation of coagulation time are as follows:

1. The blood must always be obtained under the same conditions.

2. Estimates must all be made at the same temperature.

3. The blood must always come in contact with the same amount and kind of foreign material.

[1] A preliminary report of these experiments was presented at the meeting of the American Physiological Society, Dec. 29, 1913. See Proceedings, This journal, 1914, xxxiii, p. xxxviii; also p. 372.

[2] Addis: Quarterly journal of experimental physiology, 1908, i. p. 305.

[3] Morawitz: Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1911, v, pp. 235–252.

4. The end point must be clear and definite and must always indicate the same degree of coagulation.[1]

Besides conforming to these four conditions it seemed to us that the ideal instrument should also yield a permanent objective record, made by the blood itself. The form of coagulometer finally employed is illustrated diagrammatically in Figure 1. It consists essentially of a light aluminum lever[2] with the long arm nearly counterpoised by a weight *W*. The long arm is prevented from falling by a support *S*, and is prevented from rising by a horizontal

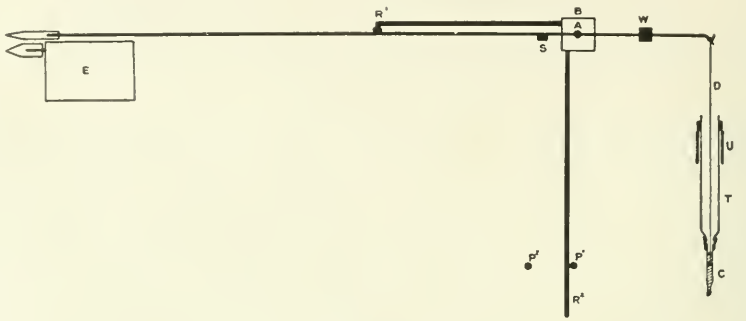

FIGURE 1. Diagram of the graphic coagulometer. The cannula at the right rested in a water bath not shown in this diagram. For further description see text.

right-angled rod reaching over the lever at R^1 and fixed into the block *B* which turns on the axis *A*. Into the same block is fixed the vertical rod R^2. When this rod is moved from the post P^1, against which it is held by the weight of the horizontal rod R^1, towards the other post P^2, the check on the long arm of the lever is lifted, and, if the short arm is heavier, the long arm will then rise.

The cannula *C*, into which the blood is received, is 2 cm. in total length and slightly more than 2 mm. in internal diameter. It is attached by a short piece of rubber tubing to the tapered glass tube *T*, 5 cm. long and 5 mm. in internal diameter. The upper end of this tube is surrounded by another piece of rubber which supports the tube when it is slid into the **U**-shaped support **U**, fixed directly below the end of the short arm of the lever.

[1] ADDIS: *loc. cit.*, p. 314.

[2] The "heart lever" made by the Harvard Apparatus Company.

By drawing the cannulas from a single piece of glass tubing and by making the distance from shoulder to upper end about 12 mm., receptacles of fairly uniform capacity are assured. All the dimensions, the reach of the rubber connection over the top of the cannula (2–3 mm.), the distance of the upper rubber ring from the lower end of the glass chamber (4 cm.), etc. — were as nearly standard as possible.

A copper wire *D*, 8 cm. long and 0.6 mm. in diameter, bent above into a hook, and below into a small ring slightly less than 2 mm. in diameter, is hung in a depression at the end of the short arm of the lever. The small ring then rests in the upper part of the cannula (see Figure 1). The weight of the copper wire makes the short arm of the lever heavier than the long arm by 30 mgm., when the delicate writing point is moving over a lightly smoked drum. Half a dozen of these standard wires were needed.

The precautions taken to fulfill the conditions stated above as essential for accurate determination of the coagulation time, were as follows:

1. Drawing the blood. — The blood was taken from the femoral artery. The artery (usually the right) was laid bare in the groin and freed from surrounding tissue. A narrow artery clip with each limb enclosed in soft rubber tubing, and with its spring exerting gentle pressure, was placed on the artery immediately below the deep femoral branch, thus allowing no blood to stagnate above the clip. Between the clip and a ligature applied about 1.5 cm. below, an opening was made. The blood was carefully milked out of the vessel between a blunt dissector moved beneath, and a small forceps, twisted into a pinch of absorbent cotton, moved above.

The cannula, cleaned in water, alcohol, and ether, was set in the rubber connection of the glass tube; the point of the cannula was then lubricated with vaseline, and slipped into the artery. The pressure of the clip on the artery was next very slightly released and blood was allowed to flow into the cannula up to the lower border of the rubber connection. Only a good-sized drop of blood was needed. Sometimes the blood ran one or two millimeters above or below, but without appreciably changing the result. Since the clip was situated on the femoral immediately

below a branch in which the circulation persisted, *the blood received in the cannula was always fresh from the moving stream.* As soon as the clip gripped the artery again, the cannula was slipped out. A helper then promptly milked the vessel in the manner described above, and covered it with a pad of absorbent cotton, smeared with vaseline, to prevent drying. Thereby blood was not permitted to stagnate; and when a new sample was to be taken, the vessel was clean and ready for use.

The tip of the cannula was at once plugged by plunging it into a flat mound of plasticine about 3 mm. high. It was drawn off sidewise lest the plasticine plug be pulled out again. One of the copper wires *D* was now slid into the tube and cannula, the tube slipped into the **U**-support, and the wire lifted and hung on the lever. This procedure, from the moment blood began to flow until the wire was hung, consumed usually about 20 seconds.

2. Uniform temperature.— Under the **U**-support was placed a large water bath, in which the cannula and the tapering part of the tube were submerged. A thermometer was fixed to the **U**-support so that the bulb came near the cannula in the bath. The water was kept within a degree of 25° C. This temperature was chosen for several reasons: (*a*) The cannula has room temperature and rapidly cools the small volume of blood that enters it. To heat blood and cannula to body temperature would take time. A bath near room temperature, therefore, seems preferable to one near body temperature. (*b*) The test of clotting was conveniently made at intervals of a half-minute, and if the clotting process were hastened by higher temperatures, this interval would become relatively less exact. (*c*) A temperature of 25° C. rather than lower was selected because, as Dale and Laidlaw [1] have shown, the coagulation time is much slower for a given change in temperature below 25° than for the same change above. And with slowing of the process the end point, when the determination depends on supporting a weight, is less likely to be sharp. (*d*) The researches undertaken with use of this coagulometer were concerned with factors hastening the process. For that reason and for reason (*b*),

[1] Dale and Laidlaw: Journal of pathology and bacteriology, 1912, xvi, p. 359.

a long rather than a short coagulation time for normal conditions was desirable.

3. Uniformity in the amount and kind of contact with foreign surface. — The capacity of the cannulas was fairly uniform, as stated above; the amount received in them was fairly constant; and the wire hanging in the blood presented approximately the same surface in different observations.

A further condition for insuring consistent treatment of the blood in different cases was that of making the tests for coagulation always at the same intervals. Below the writing point of the lever was set an electromagnetic signal E which recorded half-minutes. At the moment a record was made by the signal (see first signal mark, Figure 2), the clip on the artery was opened, the blood taken, and the process thus begun. In about 20 seconds the cannula was suspended in the water bath, and the wire was hanging on the lever. At the next record by the signal and at every subsequent record the vertical rod R^2 was pushed with the index finger from post P^1 to post P^2 and allowed to move back.[1] This motion was uniform and lasted about one second. The check R^1 on the long arm of the lever was thus raised, and as the wire sank in the blood the writing point rose, recording that coagulation had not taken place (see Figure 2).

4. Definite end-point. — As soon as the blood clotted, the weight of 30 mgm. was supported, and the failure of the lever to rise to the former height in the regular time allowed recorded that the change had occurred.

Very rarely the swing of the lever would be checked for a moment and would then begin to move rapidly, indicating that a strand of fibrin had formed but not sufficiently strong to support the weight, and that when the strand broke, the weight quickly sank in the blood. When this occurred the next record almost always was the short line, which signified that the weight was well supported.

A very slight strand of fibrin was able to prevent the weight

[1] By applying a T-shaped wire to the axis of the second hand of a clock, and bending outward at right angles the ends of the top of the T, it is possible to have the vertical rod R^2 shifted automatically at half-minute intervals. This method was not used extensively in our experiments.

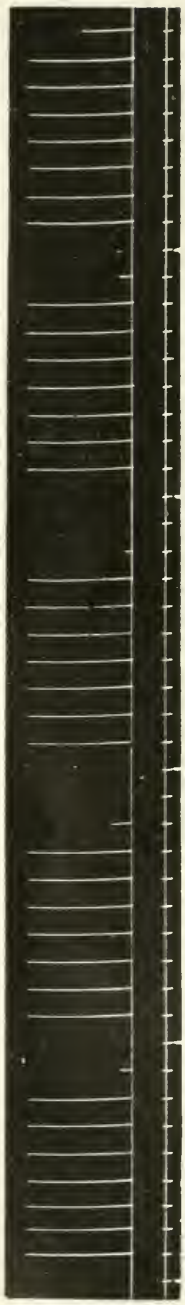

FIGURE 2. Record (reduced one-third) of five successive tests of coagulation, with the animal in a uniform condition. The lower line records intervals of 30 seconds. The marks below the time record indicate the moments when the blood samples were drawn.

from dropping, though at different times the amount of support differed, as shown by the varying length of the final lines (cf. first and last series, Figure 2). These variations are probably a rough indication of the degree of coagulation. In our experiments, however, the length of the final line was disregarded, and merely the fact that the lever failed to swing through its usual distance was taken as evidence of a clot, and the consequent short record was taken as the end point.

As soon as this end point was registered, the tube, wire and cannula were lifted out of the bath; the cannula was then separated from the tube and pulled away from the wire. The clot was thus disclosed, confirming the graphic record.

The method, at least when used at half-minute intervals, did not reveal in all instances the same degree of clotting. Usually, when the process was very rapid, the revealed clot was a thick jelly; whereas, when the process was slow, a strand of fibrin or at most a small amount of jelly was found. This difference in the *degree* of coagulation introduced, of course, an element of inexactness. In our experiments, however, this inexactness was unfavorable to the result we were seeking for, i.e., the acceleration of the process — because the jelly is a later stage than the fibrin strand; and since we nevertheless obtained good evidence of acceleration, we did not in these experiments attempt to determine more accurately differences in the stage of the clotting process.

Cleaning of apparatus. — After the wire was removed from the tube, the clot attached to its ring-tip was carefully brushed away

under cool running water. Under the running water, also, a trimmed feather was introduced into the cannula and the tube to push out the plasticine and to wash out the blood. Wire, cannula and tube were then dropped into a beaker receiving running hot water (about 80° C) and there allowed to remain for about five minutes. On removal from this the parts were shaken free from water, passed through 95 per cent alcohol and again shaken free, passed through ether and let dry.

By having a half-dozen cannulas and wires of standard size, it was possible to save trouble by cleaning a number at one time.

Not infrequently the first few samples of blood taken from an animal showed rapid or somewhat irregular rates of clotting. Some causes for these initial variations will be presented in following papers. The fairly uniform rate of clotting in any individual after the initial stage, varied in 21 different animals from an average of 3 to an average of 10.6 minutes, with a combined average of 5.9 minutes. The conditions for these variations among individuals have not been wholly determined.

The method here described has been employed not only to determine the coagulation time of cat's blood, but also that of human blood taken repeatedly through the skin at the base of the thumb nail. The skin was washed in soap and water, and rinsed in alcohol and ether before each test and then punctured with a sharp, well-pointed, three-cornered needle. The drop of blood that appeared on the skin was drawn into the cannula, and then the procedure was that above described. Following are figures obtained on one occasion:

Coagulation time	Variations from the average
5.5 minutes	+ 0.6 minutes
5 "	+ 0.1 "
4.5 "	− 0.4 "
4.5 "	− 0.4 "
5 "	+ 0.1 "
Average 4.9 minutes	Average error ± 0.3 minutes

Per cent average error = 6.

Doubtless if the lever were moved more frequently than every half-minute the average error would be reduced.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

II. The Hastening or Retarding of Coagulation by Adrenalin Injections

By W. B. CANNON and HORACE GRAY

[*From the Laboratory of Physiology in the Harvard Medical School*]

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In 1903, while tracing in dogs the course of adrenalin hyperglycaemia, Vosburgh and Richards first noted that simultaneously with the increase of blood sugar there occurred more rapid coagulation of the blood. In some cases the diminution was as much as four-fifths the coagulation time of the control. Since this result was obtained by painting adrenalin on the pancreas, as well as by intraperitoneal injection, they concluded that "the phenomenon appears to be due to the application of adrenalin to the pancreas."[1] Six years later during a study of the effect of adrenalin on internal hemorrhage, Wiggers examined incidentally the evidence presented by Vosburgh and Richards, and after many tests on five dogs found "never the slightest indication that adrenalin, either when injected or added to the blood, appreciably hastened the coagulation process."[2] In 1911, von den Velden reported that adrenalin (about 0.007 mg. per kilo) decreased the coagulation time in man about one-half — an effect appearing 11 minutes after administration by mouth, and 85 minutes after subcutaneous injection. He affirmed also, but without describing the conditions or giving figures, that adrenalin decreases coagulation time in vitro. He did not attribute the coagulative effect of adrenalin in patients to this direct action on the blood, however, but to vasoconstriction disturbing the normal circulation and

[1] Vosburgh and Richards: This journal, 1903, ix, p. 39.

[2] Wiggers: Archives of internal medicine, 1909, iii, p. 152.

thereby the normal equilibrium between blood and tissue. In consequence, the tissue juices with their coagulative properties enter the blood, so he assumes. In support of this theory he offers his observation that coagulation time is decreased after the nasal mucosa has been rendered anemic by adrenalin pledgets.[1] Von den Velden's claim for adrenalin given by mouth was subjected to a single test on man by Dale and Laidlaw, but their result was completely negative." [2]

The importance of Vosburgh and Richard's observation, the thoroughly discordant testimony of later investigators, as well as the meager and incidental nature of all the evidence that has been adduced either for or against the acceleration of clotting by adrenalin, made desirable a further study of this matter. In doing so we have employed cats as subjects. Usually they were quickly decerebrated under ether, and then continuance of the drug became unnecessary. Body temperature was maintained by means of an electric heating pad. Respiration proceeded normally except in a few instances (in which, presumably, there was hemorrhage into the medulla) when artificial respiration had to be given. The drawing of blood and the recording of coagulation were accomplished by methods already described.[3] The adrenalin used was that prepared by Parke, Davis and Co.; it was injected either subcutaneously or intravenously.

The effects of subcutaneous injections. — The first observations were of this class.

Oct. 27. A cat weighing about 3 k. was given 3 c.c. adrenalin 1: 1000, i.e., 1 mg. per kilo, under the skin. The animal, in this instance, was kept in uniform ether anaesthesia. Following is a record showing when blood was taken, and the coagulation time in each instance:

[1] Von den Velden: Münchener medizinische Wochenschrift, 1911, lviii, p. 187.

[2] Dale and Laidlaw: Journal of pathology and bacteriology, 1912, xvi, p. 362.

[3] Cannon and Mendenhall: This journal, 1914, xxxiv, p. 225.

2.56 — Injection made	3.27 — 3.5 minutes
.59 — 6 minutes	.44 — 2 "
3.07 — 5.5 "	.55 — 2.5 "
.13 — 5 "	4.07 — 3 "
.20 — 6.5 "	.20 — 2 "
Average 5.7 minutes	2.6 minutes

4.44 — 6 minutes
5.00 — 4.5 ".
5.50 — 5 "
Average 5.2 minutes

In this case the coagulation time remained at its usual level for about 20 minutes after the subcutaneous injection.[1] Thereafter for about an hour the coagulation time averaged 45 per cent of its previous duration. And widely separated tests made during the following hour indicated that approximately the initial rate of clotting had been regained.

The rather long period (nearly 30 minutes), in the case just cited, between the injection and the first appearance of rapid clotting was not the rule. As the following figures show, the coagulation time may become shortened quite promptly after subcutaneous injection, —

Oct. 29.	3.30 — 5.5 minutes	4.01 — 3.5 minutes
	.36 — 5.5 "	.08 — 3.5 "
	.44 — 3 c.c. adrenalin (1: 1000) injected	.16 — 4.5 "
	.46 — 5.5 minutes	.23 — 5 "
	.53 — 4 "	.30 — 5.5 "

In this case nine minutes after the injection the change in the rate of clotting had begun, and it continued more rapid for the subsequent half-hour.

[1] This period is longer than is expected after the subcutaneous injection of any drug. As will be shown later, *strong* doses of adrenalin injected rapidly may not at first shorten the clotting process. Probably in some instances of subcutaneous injection of these strong doses, the drug enters the circulation more rapidly than in others and in consequence coagulation is not at first accelerated.

We did not attempt to find the minimal *subcutaneous* dose which would shorten clotting. A dose of 0.01 mg. per kilo, however, has proved effective, as shown by the following figures:

Feb. 3.	11.34 — 10	minutes	
	.45 — 9	"	
	.50 to .52	"	Adrenalin, 2.8 c.c., 1: 100,000, injected under skin of groin in cat weighing 2.8 k.
	.55 — 10	minutes	
	12.06 — 7	"	
	.14 — 4	"	
	.19 — 5.5	"	
	.31 — 6	"	
	.37 — 7	"	
	.45 — 9	"	

As will be shown later, the dose in this instance was ten times the minimal effective *intravenous* dose. On the basis of these figures, less than a milligram of adrenalin given subcutaneously would be necessary to shorten clotting to a marked degree in a man of average weight (70 kg).

Not many observations were made by us on the effects of adrenalin administered subcutaneously. The amount of adrenalin reaching the vascular system and the rate of its entrance into the blood could be so much more accurately controlled by intravenous than by subcutaneous introduction that most of our attention was devoted to the latter method.

The effect of intravenous injections. — In this procedure a glass cannula was fastened in one of the external jugular veins, and filled with the same solution as that to be injected. A short rubber tube was attached and tightly clamped close to the glass. Later, for the injection, the syringe needle was inserted through the rubber and into the fluid in the cannula, the clip on the vein was removed, and the injection made.

The solutions employed intravenously were adrenalin 1: 10,000, 1:50,000, and 1:100,000 in distilled water.

The smallest amount which produced any change in clotting time was 0.1 c.c. of 1:100,000, in a cat weighting 2 kg., a dose of 0.0005 mg. per kilo. Four tests previous to the injection averaged 5 minutes, and none was shorter than 4 minutes. Immediately after the injection the time was 2 minutes, but at the next test the effect had disappeared. Doubling the dose in the same cat — i.e., giving 0.2 c.c. (0.001 mg. per kilo) — shortened the coagulation time for about 40 minutes:

Dec. 23.	10.30 — 4 minutes	11.00 — 1.5 minutes
	.35 — 4 "	.05 — 1.5 "
	.41 — 4 "	.10 — 3 "
	.46 — Adrenalin, 0.001 mg. per k.	.15 — 2 "
	.47 — 2.5 minutes	.20 — 4 "
	.50 — 3.0 "	.26 — 4.5 "
	.55 — 3.5, "	.31 — 5 "

From 10.47, immediately after the second injection, till 11.20, the average time for clotting was 2.5 minutes, whereas both before and after this period the time was 4 minutes or longer. At 11.00 o'clock and 11.05, when the end point was reached in 1.5 minutes (a reduction of 63 per cent), a thick jelly was found on examining the cannula. The changes in clotting time in this case are represented graphically in Figure 1.

FIGURE 1. Shortening of coagulation time after injecting adrenalin, 0.2 c.c. 1: 100-000 (0.001 mg. per k.), at 10.46.

In another case a dose of 0.0005 mg. per kilo failed to produce any change, but 0.001 mg. per kilo (0.28 c.c. adrenalin 1:100,000, to a cat weighing 2.8 kg.) brought a sharp decline in the record, as follows:

Jan. 9.	11.32 — 6 minutes	11.55 — 4 minutes
	.40 — 6 "	12.00 — 5.5 "
	.47 — Adrenalin, 0.001 mg. per k.	.06 — 7 "
	.48 — 5.5 minutes	

In these instances the animals were decerebrated. For decerebrate cats, the least amount of adrenalin, intravenously, needed to produce shortening of coagulation time is approximately 0.001 mg. per kilo.

In the above cases rapid clotting was manifest directly after minute doses. Larger doses, however, may produce primarily not faster clotting but slower, and that may be followed in turn by a much shorter coagulation time. The figures below present such an instance:

Nov. 25.	2.36 — 3 minutes		3.03 — 1.5 minutes
	.40 — 3 "		.05 — 1.5 "
	.43 —	Adrenalin, 0.5 c.c., 1: 10,000	.07 — 2.5 "
	.44 — 4 minutes		.10 — 1.5 "
	.49 — 3.5 "		.14 — 1.5 "
	.53 — 1.5 "		.16 — 2.5 "
	.55 — 1.5 "		.19 — 3 "
	.58 — 2 "		.23 — 3 "
	3.00 — 2.5 "		.30 — 3 "

This unexpected primary increase of coagulation time, lasting at least six minutes, is in striking contrast to the later remarkable shortening of the process from 3 to an average of 1.7 minutes for more than 20 minutes (see Figure 2, A).

If a strong solution, i.e., 1:10,000, is injected rapidly, the process may be prolonged as above, but not followed as above by shortening, thus:

Nov. 28.	9.59 — 3 minutes		10.18 — 3.5 minutes
	10.03 — 3 "		.22 — 3.5 "
	.08 —	Adrenalin, 0.5 c.c., 1: 10,000	.26 — 3 "
	.10 — 3 minutes		.29 — 3 "
	.14 — 3.5 "		.33 — 3 "

There was in this case no decrease in coagulation time at any test for a half-hour after the injection but instead a lengthening (see Figure 2, B). Howell has reported the interesting observation that repeated massive doses of adrenalin given to dogs may so

greatly retard coagulation that the animals may be said to be haemophilic.[1] These two instances show that on coagulation large doses have the contrary effect to small, just as has been shown to be true for intestinal and arterial smooth muscle.[2]

In a few experiments the brain and the cord to midthorax were destroyed through the orbit. Artificial respiration then maintained the animal in uniform condition. Under these circumstances, adrenalin intravenously had more lasting effects than when given to the usual decerebrate animals with intact cord. Figure 3 illustrates such a case. For 30 minutes before injection the clotting time averaged 5.4 minutes. Then, about 10 minutes after 1 c.c.

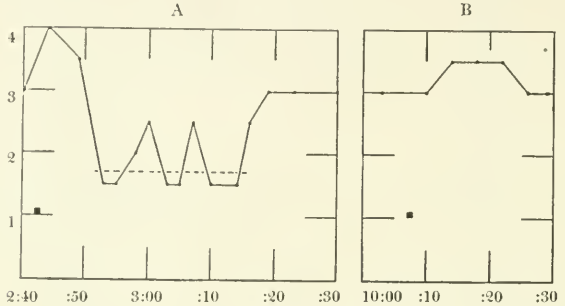

FIGURE 2. *A*, Primary lengthening followed by shortening of the coagulation time when adrenalin, 0.5 c.c. 1: 10,000 (0.05 mg.), was injected slowly, at 2.43. *B*, Lengthening of the coagulation time without shortening when the same dose was injected rapidly, at 10.08.

adrenalin 1:50,000 had been slowly injected, clotting began to quicken; during the next 20 minutes the average was 3.4 minutes, and during the following 45 minutes the average was 1.9 minutes — only 35 per cent as long as it had been before the injection.

In another case in which the brain and upper cord were similarly destroyed, the clotting time which for a half-hour had averaged 3.9 minutes was straightway reduced by 1 c.c. 1:100,000 to average for the next hour and 40 minutes 2.3 minutes, with 1.5 and 3 minutes as extremes. During the first 40 minutes of this period of 1 hour and 40 minutes of rapid clotting all of eight tests except two showed a coagulation time of 2 minutes or less.

[1] HOWELL: This journal, 1914, xxxiii, p. xiv.

[2] HOSKINS: This journal, 1912, xxix, p. 365. CANNON and LYMAN: This journal, 1913, xxxi, p. 376.

The explanation of this persistent rapid clotting in animals with spinal cord pithed is not yet clear.

As indicated in Figures 1, 2 and 3, the records of coagulation show oscillations. Some of these ups and downs are, of course, within the limits of error of the method, but in our experience they have occurred so characteristically after injection of adrenalin, and so often have appeared in a rough rhythm, that they have given the impression of being real accompaniments of faster clotting. It may be that two factors are operating, one tending to

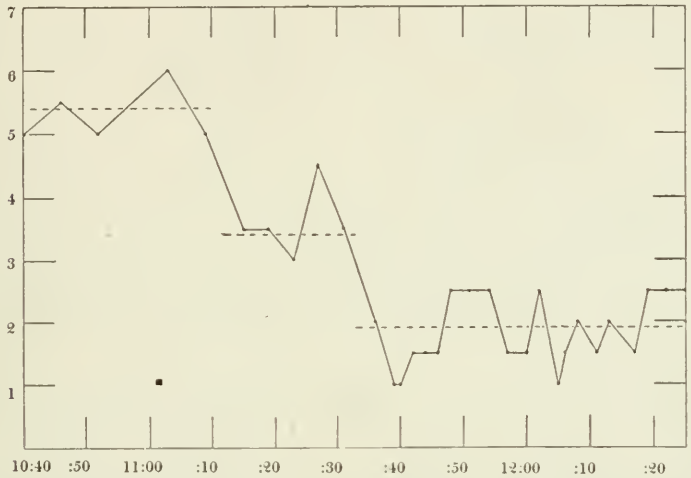

FIGURE 3. Prolonged shortening of the coagulation time after injecting (in an anima with brain and upper cord pithed) adrenalin 1 c.c. 1: 50,000 (0.02 mg.) at 11.01–02 The dash-lines represent averages.

hasten, the other to retard the process, and that the equilibrium disturbed by adrenalin is recovered only after interaction to and fro between the two factors.

The oscillations in coagulation time after adrenalin injections suggest that it might vary with changes in blood pressure, for that also commonly oscillates after a dose of adrenalin. Simultaneous recording of blood pressure and determining of coagulation time have revealed that each may vary without noteworthy variation in the other. Within ordinary limits, therefore, changes of blood pressure do not change the rate of clotting.

As previously stated, von den Velden has contended that the shortening of coagulation time by adrenalin is due to exudation of

tissue juices resulting from vasoconstriction. The least amount of adrenalin which produces markedly faster clotting in the cat, however, is 0.001 mg. per kilo. It has been shown that this amount when injected slowly, as in the present experiments, results in brief vasodilation rather than vasoconstriction.[1] Von den Velden's explanation can therefore not be applied to these experiments.

He has claimed, furthermore, that adrenalin added to blood in vitro makes it clot more rapidly, but, as already noted, he gives no account of the conditions of his experiments and no figures. It is impossible, therefore, to criticise them. His claim, however, is contrary to Wigger's earlier observations[2] that blood with added adrenalin coagulates no more quickly than blood with an equal amount of added physiological salt solution. Also contrary to this claim are the following two experiments. (1) Ligatures are tied around the aorta and inferior vena cava immediately above the diaphragm, and thus the circulation is confined almost completely to the anterior part of the animal. Indeed, since the posterior part ceases to function in the absence of blood supply, the preparation may be called an "anterior animal." When such a preparation is made and 0.5 c.c. adrenalin 1:100,000 (half the usual dose because, roughly, half an animal) is injected slowly into one of the jugulars, coagulation is not shortened. Whereas for a half-hour before the injection the clotting time averaged 4.6 minutes, for an hour thereafter the average was 5.3 minutes — a prolongation which may have been due, not to any influence of adrenalin, but to failure of the blood to circulate through the intestines and liver.[3] In another experiment after the gastro-intestinal canal and liver had been removed from the animal, the average time for coagulation during 25 minutes before injecting adrenalin (0.23 c.c. 1:100,000 in an animal weighing originally 2.3 kg.) was 5.5 minutes, and during 40 minutes after the injection it was 6.8 minutes, with no case shorter than 6 minutes. In the absence of circulation through the abdominal viscera,

[1] Cannon and Lyman: *loc. cit.*, p. 381.

[2] Wiggers: *loc. cit.*, p. 152.

[3] See Pawlow: Archiv für Physiologie, 1887, p. 458. Bohr: Centralblatt für Physiologie, 1888, ii, p. 263. Meek: This journal, 1912, xxx, p. 173.

therefore, adrenalin fails to shorten the clotting time. (2) The cannulas are filled with adrenalin, 1:1000, and emptied just before being introduced into the artery. The small amount of adrenalin left on the walls is thus automatically mixed with the drawn blood. Alternate observations with these cannulas wet by adrenalin and with the usual dry cannulas show no noteworthy distinction:

Feb. 19. 2.21 — 6.0 minutes, with usual cannula
.30 — 6.5 " " " "
.36 — 6.5 " " adrenalin cannula
.49 — 6.0 " " " "
.56 — 7.0 " " usual cannula
3.04 — 6.0 " " adrenalin cannula

The results of these experiments have made it impossible for us to concede either of von den Velden's claims, i.e., that clotting occurs faster because adrenalin is added to the blood or because adrenalin, by producing vasoconstriction, causes tissues to exude coagulant juices.

Vosburgh and Richards found that coagulation became more rapid as the blood sugar increased. Conceivably faster clotting might result from this higher percentage of blood sugar. Against this assumption, however, is the fact that clotting is greatly accelerated by 0.001 mg. per kilo, much less than the dose necessary to increase the sugar content of the blood.[1] And furthermore, when dextrose (3 c.c. of a 10 per cent solution) is added to the blood of an anterior animal, making the blood sugar roughly 0.3 per cent, the coagulation time is not markedly reduced. Adrenalin appears to act, therefore, in some other way than by increasing blood sugar.

Since adrenalin makes the blood clot much faster than normally in the intact animal, and fails to have this effect when the circulation is confined to the anterior animal, inference is justified that in the small doses here employed adrenalin produces its remarkable effects, not directly on the blood itself, nor through changes

[1] Cannon: This journal, 1914, xxxiii, p. 396.

in the extensive neuromuscular, bony, or surface tissues of the body, but through some organ in the abdomen.

That exclusion of the liver from the bodily economy by ligature of its vessels or by phosphorus poisoning,[1] will result in great lengthening of the coagulation time has been clearly shown. The liver, therefore, seems to furnish continuously to the blood a factor in the clotting process which is being continuously destroyed in the body. It is not unlikely that adrenalin makes the blood clot more rapidly by stimulating the liver to discharge this factor in greater abundance. But proof for this suggestion has not yet been established.

Summary

Adrenalin injected in small doses intravenously (0.001 mg. per kilo) and in larger doses subcutaneously, will shorten coagulation time to one-half or one-third the former duration.

The prompt shortening of the process after small doses is changed after larger doses (about 0.03 mg. per kilo) to a lengthening and later a shortening, or to a lengthening alone.

The effect of adrenalin on the clotting time is not associated with any corresponding effect on arterial pressure.

If the blood is confined anterior to the diaphragm, or if the intestines and liver are removed, adrenalin in small doses does not cause rapid clotting.

The addition of small amounts of adrenalin to drawn blood does not hasten clotting.

Increase of dextrose in the blood to 0.3 or 0.4 per cent does not cause the rapid clotting seen after adrenalin injection.

The explanation is suggested that adrenalin accelerates the clotting process by stimulating the liver (and intestines?) to greater activity in discharging some factor or factors in coagulation.

[1] See Meek: *loc. cit.*, p. 170. Also Whipple and Hurwitz: Journal of experimental medicine, 1911, xiii, p. 136.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

III. The Hastening of Coagulation by Stimulating the Splanchnic Nerves

BY W. B. CANNON AND W. L. MENDENHALL

[From the Laboratory of Physiology in the Harvard Medical School]

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IN a previous paper in this series evidence was presented that the intravenous injection of minute amounts of adrenalin hastens the clotting of blood.[1] The amounts used did not vary much above or below the amounts discharged by the adrenal glands after brief stimulation of the splanchnic nerves, as determined by Osgood in this laboratory,[2] and may therefore be regarded as physiological. Since injected adrenalin is capable of shortening the coagulation time, might not the increased secretion of the adrenals resulting from splanchnic stimulation likewise have that effect? The answer to that question was the object of the experiments here recorded.

The blood was taken and its coagulation was recorded graphically in the manner previously described.[3] In some instances the cats were etherized, in others they were anaesthetized with urethane, or were decerebrated. The splanchnic nerves always were stimulated after being cut away from connection with the spinal cord. Sometimes the nerves were isolated unilaterally in the abdomen; sometimes, in order to avoid manipulation of abdominal viscera, they were isolated in the thorax and stimulated singly or together. A tetanizing current was used, barely perceptible on the tongue and too weak to cause by spreading any contraction of skeletal muscles.

[1] Cannon and Gray: This journal, 1914, xxxiv, p. 235.

[2] See Cannon, This journal, 1914, xxxiii, p. 369.

[3] See Cannon and Mendenhall: This journal, 1914, xxxiv, p. 225.

The effects of splanchnic stimulation. — That splanchnic stimulation accelerates the clotting of blood, and that the effects vary in different animals, are facts illustrated in the following cases:

Oct. 25. A cat was etherized and maintained in uniform ether anaesthesia. After forty minutes of preliminary observation the left splanchnic nerves were stimulated in the abdomen. Following are the figures which show the effects on the coagulation time:

3.00 — 4 minutes	4.03 — 2.5 minutes
.07 — 5.5 "	.07 — 2.5 "
.14 — 4 "	.11 — 3 "
.32 — 4.5 "	.16 — 2 "
.39 to .40 Stim. left spl.	.20 — 1.5 "
.42 — 5 minutes	.23 — 4 "
.49 — 5 "	.29 — 5.5 "
.56 — 2 "	.40 — 5.5 "
4.00 — 1 "	.50 — 5 "

In this instance at least ten minutes elapsed between the end of stimulation and the beginning of faster clotting. The period of faster clotting, however, lasted for about a half-hour, during which the coagulation time averaged 2.1 minutes, only 43 per cent of the previous average of 4.8 minutes. It is noteworthy that the curve (see Figure 1), while lower, shows oscillations not unlike those which follow injection of adrenalin.[1]

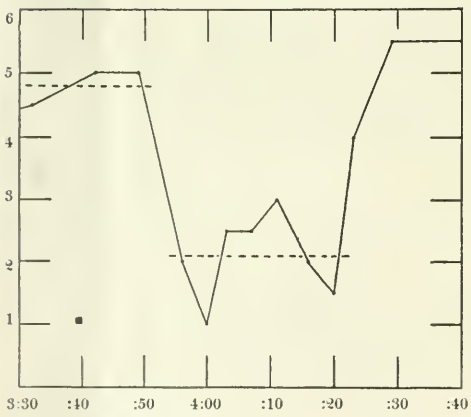

FIGURE 1. Shortening of coagulation time after stimulation of the left splanchnic nerves 3.39 — .40.

The primary delay of the effect is not always, indeed it is not commonly, present:

Nov. 6. A cat was anaesthetized (1.40 P.M.) with urethane, and later (3.05) its brain was pithed. The following observations on the

[1] Cf. CANNON and GRAY: This journal, 1914, xxxiv, p. 239.

coagulation time show the prompt effect of splanchnic stimulation:

3.36 — 7 minutes
.46 — 6 "
4.02 to .05 Stim. left spl. in abdomen
.08 — 4 minutes
.10 — 3 "
.18 — 3.5 "
.23 — 6.5 "

In Figure 2 is presented the original record of the shortening of coagulation after stimulation of the left splanchnic nerve (Nov. 8), in a cat with brain pithed.

In the foregoing instances the coagulation time was reduced after splanchnic stimulation to less than half what it was before. The reduction was not always so pronounced.[1]

Nov. 7. A cat maintained in uniform ether anaesthesia with artificial respiration had the following changes in the clotting time of its blood as the result of stimulating the left splanchnic nerve in the thorax:

3.40 — 5 minutes	4.11 — 4 minutes
.45 — 5 "	.16 — 3.5 "
.51 — 5.5 "	.21 — 4 "
.58 to 4.00 Stim. left spl.	.26 — 4.5 "
4.01 — 4.5 "	.31 — 5 "
.06 — 3.5 "	.36 — 6.5 "

In this case the average for about 15 minutes before stimulation was slightly over 5 minutes, and for 25 minutes thereafter it was four minutes.

[1] This animal had just passed through a period of excitement with rapid clotting (see CANNON and MENDENHALL: This journal, 1914, xxxiv, p.258.).

FIGURE 2. About two-fifths original size. Record of shortening of coagulation time after stimulation of the left splanchnic nerves, 4.33 — .35. The time before stimulation was 6 minutes, and afterwards 3, 4, 4, 4.5, and 6 minutes.

In all cases thus far the period of shortened coagulation lasted from 10 to 30 minutes. In other cases, however, the effect was seen only in a single observation. If this had occurred only once after splanchnic stimulation, it might be attributed to accident, but it was not an infrequent result, e.g.:

Oct. 28. A cat was etherized and decerebrated and the splanchnic nerves were isolated in the thorax. Following are two instances of brief shortening of coagulation after splanchnic stimulation:

3.36 — 4.5 minutes	4.07 — 4.5 minutes
.42 — 4.5 "	.12 — 5.5 "
.47 to .49 Spl. stim.	.19 to .22 Spl. stim.
.51 — 4.5 minutes	.23 — 3.5 minutes
.57 — 2 "	.27 — 4 "
4.01 — 4 "	.33 — 5 "

In the foregoing instance it is noteworthy that the degree of acceleration is not so great after the second stimulation of the splanchnics as it was after the first. This reduction of effect as the nerves were repeatedly stimulated was frequently noted. The following case presents another illustration:

Nov. 12. A cat was etherized (2.35 P.M.) and piqûre was performed (3.12) upon it. The operation was without effect. The loss or lessening of effectiveness on second stimulation of the left splanchnic nerves is to be compared with the persistence of effectiveness on the right side:

3.40 — 4.5 minutes	3.39 — 4 minutes
.45 — 4.5 "	.44 — 4 "
.54 to .56 Stim. left spl. in abd.	.48 — 4 "
4.00 — 3 minutes	.55 to .57 Stim. right spl.
.05 — 2 "	.59 — 3 minutes
.10 — 5.5 "	5.02 — 2.5 "
.16 — 5 "	.07 — 3 "
.22 to .27 Stim. left spl. in abd.	.11 — 3 "
.30 — 4 minutes	.15 — 5.5 "
.34 — 4 "	.22 — 5.5 "

The experiments above recorded show that stimulation of the splanchnic nerves results immediately, or after a brief period, in a

shortening of the coagulation time of the blood — an effect which in different animals varies in duration and intensity, and diminishes as the stimulation is repeated. The next question is whether this effect is produced through the adrenal glands.

The effect of splanchnic stimulation with and without the adrenal glands. — The manner in which splanchnic stimulation produces its effects is indicated in the following experiments:

Nov. 28. A cat was etherized and pithed through the orbit to the mid-thorax. The blood vessels of the *left* adrenal gland were then quickly tied and the gland removed. The readings for a half hour before the left splanchnic nerve was stimulated averaged 7 minutes, then, —

4.38 to 40 Stim. left spl. (glandless)
.42 — 7 minutes
.50 — 7 "
5.02 to .04 Stim. right spl.
.06 — 4 minutes
.10 — 7 "
.18 — 7 "
.26 — 7 "

Dec. 4. A cat was etherized and pithed through the orbit to the neck region. The right and left splanchnic nerves were tied and cut in the thorax. The *left* adrenal gland was then carefully removed. These operations consumed about a half-hour. The following records show the effect of stimulating the left and right splanchnic nerves:

4.10 — 5 minutes
.16 — 4.5 "
.25 to .28 Stim. left spl. (glandless)
.30 — 4.5 minutes
.35 — 4.5 "
.40 — 7.5 "
.49 to .51 Stim. right spl.
.55 — 4.5 minutes
5.00 — 2.5 "
5.14 — 6 minutes
.23 to .25 Stim. right spl.
.26 — 6 minutes
.33 — 4.5 "
.38 — 3.5 "
.43 — 4.5 "
.49 — 5 "
.55 — 6 "

The results in this experiment are represented graphically in Figure 3.

Elliott has presented evidence that in the cat the splanchnic innervation of the adrenals is not crossed, so that if the gland is removed on one side stimulation of the nerves on that side causes no discharge from the opposite gland.[1] As the above experiments clearly show, splanchnic stimulation on the glandless side results in no shortening of the coagulation time; whereas, in the same

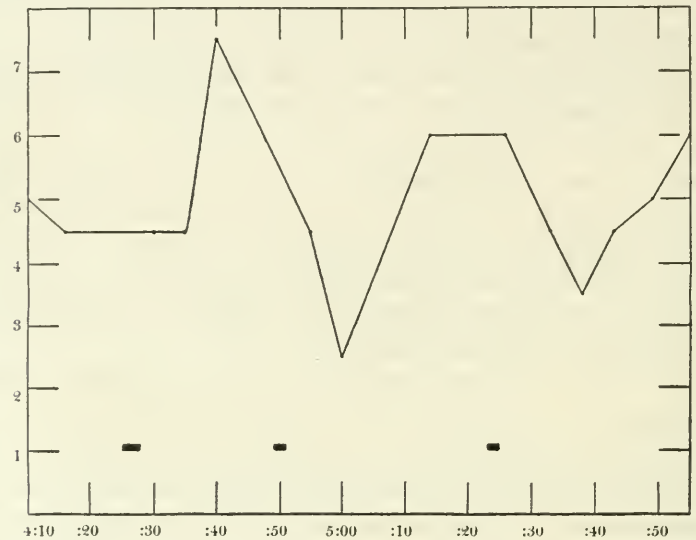

FIGURE 3. Results of stimulating the left splanchnic nerves, 4.25 — 28, after removal of the left adrenal gland, and of stimulating the right splanchnic nerves, 4.49 — .51 and 5.23 — .25, with right adrenal gland present.

animals, stimulation of the nerves on the other side (still connected with the adrenal gland) produces a sharp hastening of the clotting process.

The splanchnics innervate the intestines and liver even though the adrenal gland is removed. The foregoing experiments indicate that the nerve impulses delivered to these organs do not influence them in any direct manner to accelerate the speed of coagulation. Indeed in one of the experiments (Dec. 4, p. 247) a high reading about ten minutes after splanchnic stimulation on the glandless side suggests the possibility of an opposite effect. Direct stimulation

[1] ELLIOTT: Journal of physiology, 1912, xliv, p. 405.

of the hepatic nerves on one occasion was followed by a change of the clotting time from 4.5, 5, 4.5, 4.5 minutes during 25 minutes before stimulation to 4.5, 7, and 6 minutes during 20 minutes after stimulation.

Since with the adrenals present stimulation of hepatic nerves induces glycogenolysis and quick increase of blood sugar,[1] just as splanchnic stimulation does, the failure of the blood to clot faster after stimulation of the hepatic nerves confirms the evidence already offered that faster clotting in hyperadrenalinaemia is not due to hyperglycaemia.[2]

The liver and intestines cannot be made to shorten clotting time by stimulation of their nerves, but, as has already been shown, neither can adrenalin act by itself to hasten the clotting process.[3] Apparently the effect is produced by coöperation between the adrenals and the liver (and possibly also the intestines). Somewhat similar cooperation is noted in the organization of sugar metabolism; splanchnic stimulation in the absence of the adrenal glands does not increase blood sugar,[4] and in the absence of the liver adrenalin is without influence.[5]

The variations of effect noted after splanchnic stimulation can be accounted for by variations in the adrenalin content of the glands. Elliott reports that animals newly brought into strange surroundings may have a considerably reduced amount of adrenalin in their adrenals.[6] The animals used in our experiments had been for varying lengths of time in an animal house in which barking dogs were also kept, and were therefore subject to influences which would be likely to discharge the glands.

[1] Macleod: Diabetes: its Pathological Physiology, London, 1913, pp. 68–72.

[2] See Cannon and Gray: This journal, 1914, xxxiv, p. 241.

[3] See Cannon and Gray: *loc. cit.*, p. 240.

[4] Gautrelet and Thomas: Comptes rendus de la Société de Biologie, 1909, lxvii, p. 233.

[5] Bang: Der Blutzucker, Wiesbaden, 1913, p. 87.

[6] Elliott: *loc. cit.*, p. 379.

Summary

Stimulation of the splanchnic nerves results immediately, or after a brief delay, in shortening of the coagulation time of blood. The degree and the duration of the effect varies, — clotting not uncommonly takes less than half the time it took before stimulation, and this period of rapid clotting may last from 10 to 30 minutes.

The stimulation usually produces less marked effects as it is repeated.

If the adrenal gland is removed on one side, splanchnic stimulation on that side does not shorten the clotting time; whereas splanchnic stimulation on the other side is still effective. The faster clotting is therefore due to increased adrenal discharge.

Since stimulation of nerves supplying the liver and intestines does not hasten clotting, and since increase of adrenalin has no effect in the absence of the liver and intestines, the shortened clotting after splanchnic stimulation is accounted for by the action of adrenal discharge on the liver (and intestines?).

The variations in the effects in different animals can be accounted for by variations in the adrenalin content of the adrenal glands in confined animals.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

IV. The Hastening of Coagulation in Pain and Emotional Excitement

By W. B. CANNON and W. L. MENDENHALL

[*From the Laboratory of Physiology in the Harvard Medical School*]

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IN the preceding paper of this series evidence was given to prove that stimulation of splanchnic nerves, with accompanying increase of adrenal secretion, results in more rapid clotting of blood. Recent experiments have shown that certain conditions — such as pain and emotional excitement — likely to arise in the natural life of organisms and known to be attended by nervous discharges over splanchnic courses, are also attended by increased secretion of adrenalin into the blood.[1] Does the adrenalin thus liberated have any effect on the rate of coagulation? The observations here recorded were made in order to obtain an answer to that question.

The effect of "painful" stimulation. — In experiments on the action of stimuli which in the unanaesthetized animal would cause pain, faradic stimulation of a large nerve trunk (the stump of the cut sciatic), and operation under light anaesthesia, were the methods used to affect the afferent nerves. Elliott found that repeated excitation of the sciatic nerve was especially efficient in exhausting the adrenal glands of their adrenalin content, and also that this reflex persisted after removal of the cerebral hemispheres.[2] It was to be expected, therefore, that with well-stored glands, sciatic stimulation, even in the decerebrate animal, would call forth an amount of adrenal secretion which would decidedly hasten clotting. The following case illustrates such a result:

[1] Cannon: This journal, 1914, xxxiii, p. 357.

[2] Elliott: Journal of physiology, 1912, xliv, pp. 406, 407.

Dec. 12. A cat was anaesthetized with ether at 3.45, and the left sciatic nerve was bared. Decerebration was completed at 3.57. The clotting time of the blood began to be tested six minutes later:

4.03 — 4 minutes	4.45 to .50 Stim. left sciatic
.08 — 3.5 "	.53 — 2.5 minutes
.13 — 3.5 "	.57 — 7 "
.18 — 4.5 "	5.06 — 7.5 "
.23 to .25 Stim. left sciatic	.15 to .17 Stim. left sciatic
.26 — 2.5 minutes	.17 — 4 minutes
.29 — 3.5 "	.22 — 4.5 "
.34 — 4.0 "	.27 — 5.5 "
.40 — 5.0 "	.36 — 5.5 "
	.46 — 7 "

The results obtained in this case, which were similar to results in other cases, are represented graphically in Figure 1. The

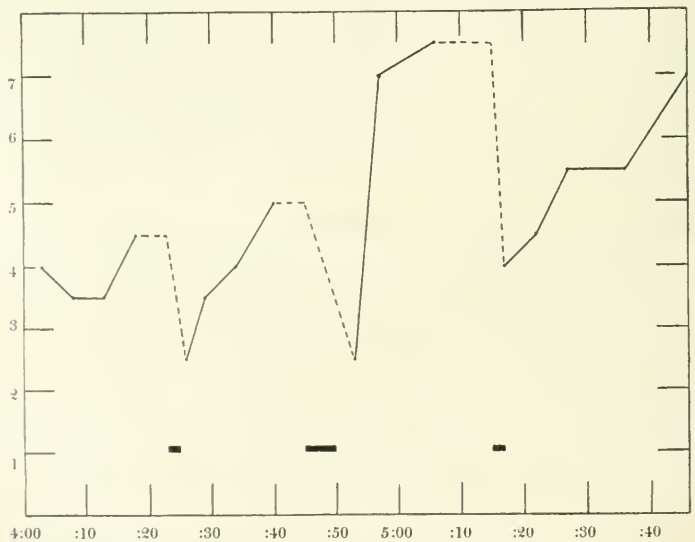

FIGURE 1. Three shortenings of coagulation time after stimulation of the left sciatic nerve, at 4.23 — .25, at 4.45 — 50 (stronger), and at 5.15 — .17.

coagulation time was becoming gradually more prolonged, but each excitation of the sciatic nerve was followed by a marked shorten-

ing. The strength of stimulation was not determined with exactness, but it is worthy of note that the current used in first and third stimulations was weaker than could be felt on the tongue, whereas that used in the second was considerably stronger, though it did not produce reflex spasms.

Mere tying of the nerve is capable of producing a marked shortening of coagulation, as the following figures show:

Oct. 21. 10.57 Cat under ether and urethane given
11.11 — 8.5 minutes
.23 — 8.5 "
.32 to .35 Left sciatic bared and tied
.37 — 1.5 minutes
.41 — 5.5 "
.50 — 7 "
12.02 — 8.5

Stimulation of the crural nerve had similar effects, reducing the clotting time in one instance from a succession of 3, 3, and 3.5 minutes to 1.5 minutes shortly after the application of the current, with a return to 3.5 minutes at the next test.

Operative procedures performed under light anaesthesia, or reduction of anaesthesia soon after operation, resulted in a remarkable shortening of the coagulation time:

Nov. 8. A cat was etherized and tracheotomized. The abdomen was then opened, and a ligature was drawn around the hepatic nerves. The operation was completed at 2.25. At 2.50 the etherization became light and the rate of clotting began to be faster:

2.50 — 6 minutes	.15 — 3.5 minutes
3.00 — 5.5 "	.20 — 4.5 "
.10 — 3.5 "	.30 — 7.5 "

Nov. 11. A female cat, very quiet, was placed in the holder at 1.55. The animal was not excited. At 2.10 etherization was begun; the animal was then tracheotomized, and the femoral artery was exposed.

2.21 — 4.5 minutes

.26 — 4.5 " Anaesthesia lessened

.32 — 3.5 " " light

.35 — Abdomen opened

.47 — 1.5 minutes

.52 — 1 "

.55 — Ligature passed around hepatic nerves

.57 — 1.5 minutes Anaesthesia light; corneal reflex present

3.02 — 3 "

.07 — 3 " Some hepatic nerves cut

.12 — 4.5 " Rest of hepatic nerves cut

.22 — 5 "

The results of this experiment are shown graphically in Figure 2.

Nov. 13. A cat was etherized at 1.55, tracheotomized, and the femoral artery laid bare. As soon as these preparations were completed, the ether was removed and anaesthesia became light. The blood clotted thus:

2.08 — 6 minutes,

.15 — 4 " Anaesthesia light

.20 — 2 "

.24 — 1 " Etherization begun again

.27 — 2.5 "

.30 — 3.5 "

.35 — 5.5 "

.50 — 5.5 "

In the foregoing and in other similar instances, a condition of surgical injury, whether just made or being made, was accompanied by more rapid clotting of blood when the degree of anaesthesia was lessened. This condition was one which, if allowed to go further in the same direction, would result in pain. Both direct electrical stimulation and also surgical operation of a nature to

give pain in the unanaesthetized animal, result, therefore, in faster clotting. It is worthy of note that after decerebration clotting apparently occurred no faster because the abdomen had been opened, although in the decerebrate state etherization was suspended. The mechanism for reflex control of the adrenals may not be higher than the corpora quadrigemina, as Elliott has shown, but the discharge from the glands seems to be more certain to occur when the cerebrum is present and is permitted even slightly to operate.

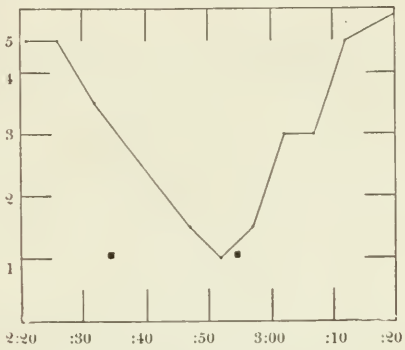

FIGURE 2. Shortening of coagulation time during an operation under light anaesthesia. At 2.35 the abdomen was opened, at 2.55 a ligature was passed around the hepatic nerves.

The effect of emotional excitement. — Reference has already been made to the emotional secretion of the adrenal glands. In their emotional reaction to being bound cats differ widely: some, especially young males, become furious; others, especially elderly females, take the experience quite calmly. This difference of attitude was used with positive results in experiments on emotional glycosuria,[1] and it seemed possible, therefore, to use it to test the effect of emotions on blood-clotting. To plan formal experiments for that purpose was not necessary, because in the ordinary course of the researches here reported, the difference in effects on the blood between the violent rage of vigorous young males and the quiet complacency of old females was early noted. Indeed the rapid clotting which accompanied excitement not infrequently made necessary an annoying wait till slower clotting would permit the use of experimental methods for shortening the process.

The animals used on November 11 and 13 (see pp. 253, 254) are examples of calm acceptance of being placed on the holder, and furthermore these animals were anaesthetized without much disturbance. As the figures indicate, from the first the clotting occurred at about the average rate.

[1] See CANNON, SHOHL, and WRIGHT: This journal, 1911, xxix, p. 280.

In sharp contrast to these figures are those obtained when a vigorous animal is angered:

Oct. 30. A very vigorous cat was placed on the holder at 9.08. It at once became stormy, snarling, hissing, biting, and lashing its big tail. At 9.12 etherizing was begun and that intensified the excitement. By 9.15 the femoral artery was tied. The clotting time of the blood for an hour after the ether was first given was as follows:

9.18 — 0.5 minute	9.43 — 1.0 minute
.19 — 1.0 "	.45 — 0.5 "
.22 — 1.0 "	.49 — 0.5 "
.24 — 1.0 "	.52 — 0.5 "
.26 — 1.0 "	.54 — 0.5 "
.28 — 1.5 "	.57 — 1.0 "
.31 — 1.0 "	10.00 — 0.5 "
.33 — 0.5 "	.02 — 0.5 "
.35 — 0.5 "	.06 — 1.0 "
.38 — 0.5 "	.09 — 0.5 "
.39 — 0.5 "	.11 — 0.5 "
.41 — 1.0 "	.13 — 1.0 "

Twenty-four observations made during the hour showed that the clotting time in this enraged animal averaged three-fourths of a minute and was never longer than a minute and a half. The clots were invariably a solid jelly. The persistence of the rapid clotting for so long a period after anaesthesia was started, may have been in part due to continued, rather light etherization, for Elliott found that etherization itself could reduce the adrenalin content of the adrenal glands.[1]

The shortened clotting did not always persist so long as in the foregoing instance. The brief period of faster clotting illustrated in the following case was typical of many:

Nov. 18. A cat that had been in stock for some time was placed on the holder at 2.13, and was at once enraged. Two minutes later

[1] Elliott: *loc. cit.*, p. 388.

etherization was started. The hairs on the tail were erect. The clotting was as follows:

2.25 — 1.0 minute	.31 — 4.5 minutes
.27 — 0.5 "	.37 — 3.5 "
.28 — 2.0 "	.47 — 4.5 "

It seems probable that in this case, because of the cat's being caged near dogs, the adrenals were well-nigh exhausted previous to this experiment, and that the emotional flare-up practically discharged the glands, for repeated attempts later to reproduce the initial rapid clotting by stimulation of the splanchnic nerves was without result.

Evidence presented in previous papers of this series makes wholly probable the correctness of the inference that the faster coagulation which follows emotional excitement is due to adrenal discharge from splanchnic stimulation. In this relation the effect of severance of the splanchnics on emotional acceleration of the clotting process is of interest. The following cases are illustrative:

Oct. 29. A cat was left on the holder for 10 minutes while the femoral artery was uncovered under local anaesthesia. The blood removed was clotted in a half-minute. The animal was much excited. It was now quickly etherized and the brain pithed forward from the neck. The tests resulted as follows:

10.51 — 1.0 minute	
.53 — 05. "	
.55 — 0.5 "	
.57 — 0.5 "	
11.07 —	Cut left splanchnic
.12 —	" right splanchnic
.21 — 3.5 minutes	
.26 — 3.5 "	

The original record of this case is given in Figure 3.

Nov. 5. A cat was etherized at 2.35. At 2.39 artificial respiration by tracheal cannula was begun, the air passing through an ether bottle. The clotting occurred thus:

2.53 — 1.5 minutes
.57 — 1.5 "
3.05 — 1.5 "
.15 — 1.5 "
.25 — Both spl. cut and tied in thorax
.35 — 4.5 minutes
.55 — 4.5 "

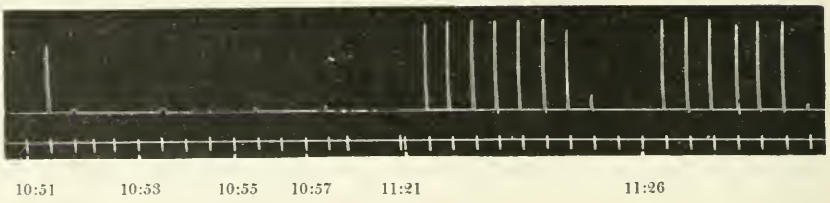

FIGURE 3. About two-thirds original size. Record of rapid clotting (less than a half-minute) after emotional excitement. At 11.07 the left, at 11.12 the right splanchnic nerves were cut; the clotting then required 3.5 minutes. The marks below the time record indicate the moments when the samples were drawn.

Nov. 7. A cat was etherized under excitement and with tail-hairs erect, at 1.55. At 2.13 the animal was showing reflexes. The figures show the course of the experiment:

2.15 — 1.5 minutes
.21 — 1.0 "
.26 — 1.0 "
.31 — 1.0 "
.36 — 1.0 "
.41 — 1.0 "
.46 — 2.0 "
.51 — 2.0 "
3.06 — 2.0 minutes
.11 — 2.5 "
.26 — Cut left spl. in thorax
.35 — " right spl. in thorax
.40 — 5.0 minutes
.45 — 5.0 "
.51 — 5.5 "

In this instance the subsequent stimulation of the splanchnic nerves resulted again in faster clotting — a reduction from 5.5 minutes to 3.5 minutes.[1] The results from this experiment are shown graphically in Figure 4.

[1] See CANNON and MENDENHALL: This journal, 1914, xxxiv, p. 245, experiment on Nov. 7.

Discussion

The data presented in this paper show that such stimulation as in the unanaesthetized animal would cause pain, and also such emotions as fear and rage, are capable of greatly shortening the coagulation time of blood. These results are quite in harmony with the evidence previously offered that injected adrenalin and

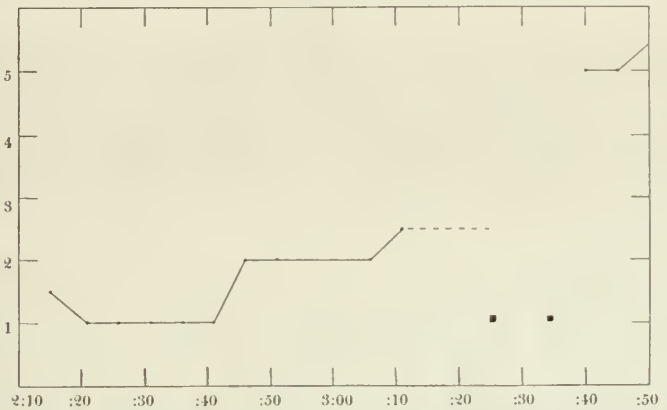

Figure 4. Rapid clotting after emotional excitement, with slowing of the process when the splanchnic nerves were cut in thorax (the left at 3.26, the right at 3.35).

secretion from the adrenal glands induced by splanchnic stimulation hasten clotting, for, as already stated, painful stimulation and emotional excitement also evoke activity of the adrenals.

In a previous paper the increase of blood sugar and the secretion of adrenalin in pain and the major emotions were interpreted as biological adaptations to conditions likely to involve, in wild life, pain and great emotion — i.e., the necessities of struggle, fighting or flight. The sugar would then serve the muscular energies, the adrenalin would aid in distributing blood to organs critically involved in the struggle, and would also abolish or minimize the effects of fatigue.[1] The more rapid clotting of blood in pain and emotional excitement may also be regarded as an adaptive process, useful to the organism. The importance of conserving the blood needs no argument. The effect of local injury in favoring the formation of a clot to seal the opened vessels

[1] See Cannon: This journal, 1914, xxxiii, p. 372.

is obviously adaptive in protecting the organism against hemorrhage. The injury that causes opening of blood vessels, however, is, if extensive, likely also to produce pain. And, as shown above, conditions producing pain increase adrenal secretion and hasten coagulation. Thus injury would be made less dangerous as an occasion for serious hemorrhage by two effects which the injury itself produces in the body, — the local effect on clotting at the region of injury and the general effect on the speed of clotting wrought by reflex secretion of adrenalin.[1]

The strong emotions, as fear and anger, are reasonably regarded as the concomitants of bodily changes which may be of utmost service in subsequent action. These bodily changes are so much like those which occur in pain and fierce struggle that, as early writers on evolution suggested, the emotions may be considered as foreshadowing the suffering and intensity of actual strife. On this general basis, therefore, the bodily alterations attending violent emotional states would, as organic preparations for fighting and possible injury, naturally involve the effects which pain itself would produce. And rapid clotting, like increased blood sugar, increased adrenalin, and an adapted circulation, would be favorable to the preservation of the organism that could best effect it.[2]

[1] The conditions under which pain and danger from hemorrhage occur together in civilization are not so frequent as to permit a ready testing on man of the ideas here propounded. It is possible that the pain of childbirth is such as to lead to adrenal discharge (the increase of blood-sugar during parturition is indicative of that), and thus to favor rapid clotting at a time when that may be important.

[2] There is evidence that asphyxia causes increased secretion of adrenalin (see CANNON: This journal, 1914, xxxiii, p. 357), and often in the course of these investigations we have noted that when respiration became impaired and the blood turned dark, clotting was faster. In a few observations, however, in which a formal attempt was made to determine the influence of asphyxia on clotting, the results were not always positive — due probably to the use of animals which had been kept for some time in conditions likely to discharge the adrenal glands.

Stewart (Journal of experimental medicine, 1912, xv, p. 547) and Hoskins and McPeek (Journal of the American Medical Association, 1913, lx, p. 1778) have reported that direct massage of the adrenal glands causes an increased secretion of adrenalin. The increase, according to the latter authors, is slight. Mr. Horace Gray and Mr. C. A. L. Binger have tried in this laboratory the

Summary

Stimulation of afferent nerves (sciatic, crural), or major operations under light anaesthesia, markedly shorten the coagulation time of blood.

Emotional excitement is the occasion for very rapid clotting (sometimes in less than a half-minute), which becomes slow (three to five minutes) when the splanchnic nerves are cut.

Pain and strong emotions have been proved to evoke secretion of the adrenal glands; and adrenalin hastens clotting. Rapid coagulation may reasonably be considered, therefore, as another instance of adaptive reaction serviceable to the organism in the injury which may accompany pain or which may follow the struggle that fear or rage may occasion.

possibility of influencing the coagulation of blood by massage of the adrenals. In one instance the clotting time was 5.5 minutes in four successive tests before massage. After massage it fell to an average of 4.6, for forty minutes. Then the glands were manipulated again, and the eight tests taken thereafter were as follows: 3.5, 4.5, 4, 4, 3.5, 4.5, 3.5, 4. In another instance massage of the glands reduced the time, after a brief prolongation, from an average of 6.1 minutes to an average of 4.7 minutes, with six tests in each group. In other cases the tests were not so favorable. Massage of the liver was without clear effect.

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PARATHYROID DEFICIENCY AND SYMPATHETIC IRRITABILITY

By R. G. HOSKINS and HOMER WHEELON

[From the Laboratory of Physiology of the Northwestern University Medical School.]

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IN the further development of the physiology of internal secretion a clean-cut knowledge of the relation of the various endosecretory organs to the sympathetic nervous system seems particularly desirable. In the hope of throwing additional light upon the subject a series of researches of which this is the third has been undertaken in this laboratory.[1]

There are to be found in the literature but few observations bearing upon the relation of the parathyroid glands to the sympathetic system. Falta and Kahn[2] in 1912 studied the effects of parathyroid extirpation in a dog. Both parathyroids were removed on one side leaving the thyroid lobe. On the other side the thyroid lobe with the two parathyroids was removed. Later blood pressure was recorded and the reaction to epinephrin in various dilutions determined. A *depression* was observed with quantities of the drug which in the normal animal give a *pressor* effect. The significance of the observation is not clear. It is interpreted by Falta and Kahn as indicating an increased sympathetic irritability. In view of the fact, however, that the first reaction of a dog to

[1] Hoskins and Wheelon: This Journal, 1914, xxxiii, pp. 81, 172.

[2] Falta and Kahn: Zeitschrift für klinische Medicin, 1912, lxxiv, p. 108.

ascending dosages of epinephrin is depression and that this reaction was relatively late in appearing, their results would seem rather to indicate a lowering of sympathetic irritability following parathyroidectomy. In the same article Falta and Kahn report also the results of a series of studies of cases of clinical tetany. In these the patients characteristically showed an augmented irritability to epinephrin as judged by increase of blood pressure or pulse rate. In the absence of definite proof that the tetanies observed were due to parathyroid deficiency their findings are not conclusive as regards the subject of this paper. An increased sensitiveness to pilocarpin was also observed. It was concluded, therefore, that in parathyroid deficiency there is an augmented irritability of the whole autonomic system.

Lately there have been published from Carlson's laboratory several papers which deal with parathyroid deficiency. In 1912 Carlson [1] concluded from a study of the activities of the digestive tract that parathyroid extirpation causes both in the cat and in the dog a depression of the sympathetic system. Although in the final stages of tetany in his cats a marked depression of the motor function of the stomach and intestines was observed, in most instances the animals in this respect were normal. In dogs, however, both the motor and secretory activities deviated in the direction of depression. On the face of it the evidence would seem to indicate an overactivity of the splanchnic sympathetics, but Carlson interprets it otherwise. Salivation was noted as a common symptom, but this was ascribed to bulbar autonomic rather than sympathetic influences. Other observations were recorded upon mydriasis, sweating, bladder tonus, defecation and parturition, some of which suggest abnormal sympathetic activity, but which as a whole were regarded as indicating sympathetic depression. In a later paper Carlson [2] records that during attacks of tetany after thyro-parathyroidectomy there is a marked loss of gastric muscular tonicity; during the interim between attacks the tonus returns to normal. Similarly Keeton [3] has found a depression of gastric secretion, a decreased hydrogen ion and probably decreased pepsin out-

[1] Carlson: This Journal, 1912, xxx, p. 309.
[2] Carlson: *Ibid.*, 1913, xxxii, p. 398.
[3] Keeton: *Ibid.*, 1914, xxxiii, p. 25

put which appears as parathyroid tetany develops. Stoland [1] has noted a depression of bile and of pancreatic juice under similar conditions. These observations, all of which suggest excessive sympathetic functioning, are interpreted in harmony with Carlson's first conclusions as due to other causes.

In our experiments blood pressure was used exclusively as a criterion of sympathetic conditions. The comparative irritability of the sympathetic system before and after parathyroid destruction was determined by injecting fixed quantities of nicotin and of "adrenalin." To test the condition of the muscular tissues involved pituitrin was also used in several cases. In all the experiments dogs were employed. The technique in general was the same as that of the preceding investigations of the series. The animals were anesthetized with ether. Then with aseptic precautions a reservoir cannula was inserted into a femoral artery for taking blood-pressure records and a simple large-bore cannula into the corresponding vein for the injecting of fluids. The standard doses of drugs employed were usually adrenalin 0.5 c.c. and 1.0 c.c. 1:50,000 dilution, nicotin 0.5 c.c. and 1.0 c.c. 1:2,000 and pituitrin 0.1 c.c. In case an animal was small or showed unusual irritability the dosage was correspondingly diminished. With a view to its subsequent use the reaction to 1 gm. of calcium lactate given intravenously was also determined. The condition of the vasomotor system having been established, the opened blood vessels were tied off and the incision closed and sutured.

Immediately preceding or following the blood-pressure determinations the thyroid glands were exposed by bringing them out through a median incision. An attempt was then made to identify all four parathryoid glands. In several cases the attempts were successful. All the glands found were destroyed by cautery. In case both glands were found on one side only, the thyroid of the opposite side was removed with its capsule so as to include both parathyroids. In other instances where only posterior parathyroids were found these were cauterized and the anterior half of each thyroid removed so as to include the anterior parathyroids. Asher and Rodt [2] have concluded that thyroid secretion exerts a considerable

[1] Stoland: *Ibid.*, 1914, xxxiii, p. 283.

[2] Asher und Rodt: Centralblatt für Physiologie, 1912, xxvi, p. 223.

effect upon the sympathetic system. In nearly all cases, therefore, care was taken to leave sufficient thyroid tissue for normal functioning, but in two animals complete removal of all demonstrable thyroid and parathyroid tissue was made. Aseptic technique of course was employed. The incisions were closed and the animals kept from one to five days.

Blood pressures and the reactions to "adrenalin," nicotin, and pituitrin were then taken with the same dosages as before, using the blood vessels of the other hind leg corresponding to those used in the first determinations. In case a third determination was made the cannulas were inserted in one or the other of the femoral vessels proximal to the former incision. In several instances 1 gm. of calcium lactate was given intravenously and the drug injections repeated.

In only one case (No. 62) in which the animals survived (aside from one nearly moribund of hemorrhage) did we fail to get clear evidence of parathyroid deficiency. Nearly all the animals developed pronounced tetany. In one, however, the only evident sign was an augmentation of reflexes. A light tap on the paw or hip would result in a quick jerk of the leg. Otherwise the dog showed a disinclination to move. In the case of the animal (No. 62) in which no signs of parathyroid deficiency were detected an apparently normal gland was found at autopsy; this therefore is not a valid negative case. Of all the animals showing external evidence of deficiency, only one failed to show also an augmented sympathetic irritability. The blood-pressure determination was made in this case 24 hours after the first operation. A later determination was not made. So far as it goes this observation indicates that augmentation of irritability develops in the sympathetic system more slowly than in the voluntary neuromuscular apparatus.

Figure 1 shows the results secured with adrenalin in case of dog No. 60. (*a*) shows the reaction to 0.5 c.c., 1:50,000. The parathyroids were then removed. (*b*) shows the reaction to the same quantity of adrenalin two days later. One gram of calcium lactate was given intravenously and a few minutes later the reaction (*c*) again determined.

Figure 2 (*a*) shows the effect in dog No. 55 of 0.5 c.c. of nicotin, 1:2000, before the operation and 2 (*b*) the effect of the same dosage two days after parathyroidectomy.

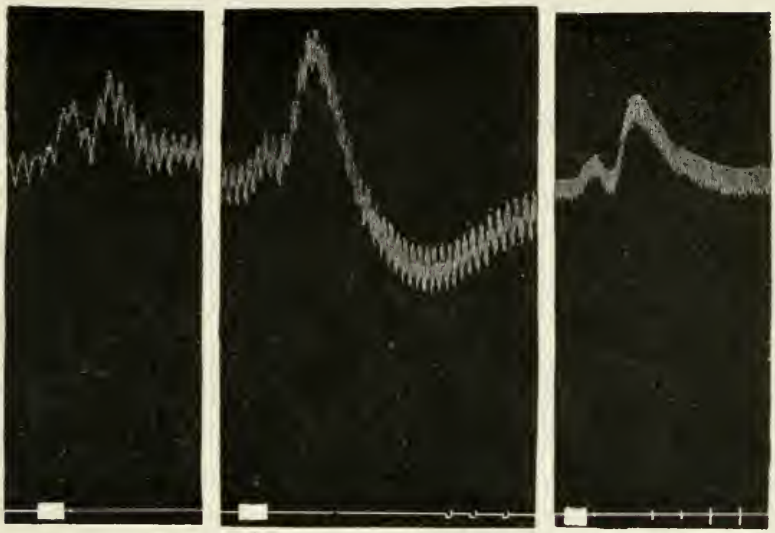

FIGURE 1. (*a*) Reaction in normal dog to 0.5 cc. "adrenalin," 1:50,000. (*b*) Reaction to same dosage in same dog 2 days after parathyroid destruction. (*c*) Reaction to same dosage after 1 gm. calcium lactate intravenously. Blood pressure from femoral artery.

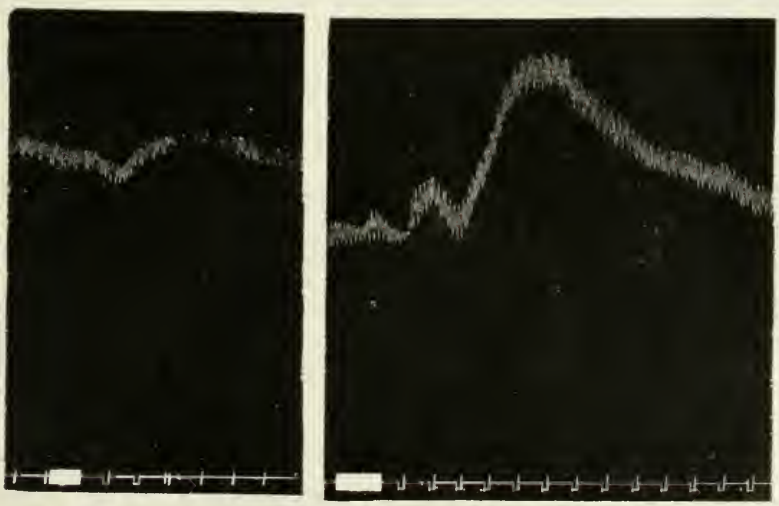

FIGURE 2. (*a*) Reaction in normal dog to 0.5 cc. Nicotin 1:2,000. (*b*) Reaction in same dog to same dosage 2 days later. after destruction of parathyroid glands. Blood pressure from femoral artery. Time 5 sec.

Dog No. 68 exemplified the trend of the investigation as a whole. The protocol is as follows:

Old female coach dog. Wt. 8.9 Kilos.

March 19, 1914, 9:50 A.M. Animal etherized. Cannulas inserted into right femoral artery and vein.

10:15–10:35 Reactions determined to nicotin 0.3 and 0.5 c.c. 1-2000, adrenalin 0.5 c.c., 1:50,000 (5-minute intervals).

10:40 Injected calcium lactate 1 gram.

10:50–11 Reactions to nicotin 0.5 c.c. 1:2000, adrenalin 0.5 c.c. 1:50,000. Reactions slightly greater than before calcium.

11:10–11:40 Four parathyroids demonstrated and cauterized. Thyroids slightly larger than normal, parathyroids apparently normal.

March 21, 10 A.M. Clonic convulsions observed. Animal otherwise appeared normal.

12:00 M. Fine tremors throughout body but especially in limbs. No clonus.

1:30 P.M. Condition same as at noon.

1:50 Etherized. Fine tremors persist. No clonus. Animal vomited during operation of inserting cannulas.

2:06–2:20 Reactions to nicotin and adrenalin as before. Reactions slightly increased above normal.

March 23, 2:30 P.M. Fine tremors.

March 24, 10:00 A.M. Clonic convulsions and tremors throughout body.

1–2:30 P.M. Slight tremor, particularly in shoulder muscles. No clonus.

2:30–3:50 Occasional clonus of different muscles, gradually becoming more frequent. Reflexes in legs (mechanical stimulus) augmented.

3:50 Etherized. Cannulas inserted, femoral vessels.

4:12–4:25 Reactions to nicotin 0.3 c.c., 0.5 c.c., adrenal 0.5 c.c. as before. Reaction to nicotine markedly increased. To adrenalin very slightly decreased.

4:26 Calcium lactate 1 gram.

4:30–4:55 Nicotin 0.5 c.c. at 5-minute intervals. Reaction gradually decreased to 4:45 then increased nearly to previous level.

Animal killed.

Autopsy: Thyroids normal. No parathyroid tissue discoverable. Scars of four cauterizations.

In view of Carlson's observation that loss of gastric tonicity occurs only during attacks of frank tetany with return approximately to normal in the interim we rather expected to find a parallelism between the severity of external symptoms and the degree of vasomotor irritability. The researches as a whole, as in case of No. 68, have given the impression that to some extent this is true, but various exceptions were noted. No. 55, for example, showed as great augmentation of vasomotor irritability at the third determination when there was complete absence of clonus as in the second when clonus was severe. No. 63, 24 hours after operation, showed pronounced clonus and tremor with no augmentation of vasomotor irritability, while No. 65 in an interval of freedom from clonus and tremors after an attack of severe tetany showed augmented sympathetic irritability.

The reactions to pituitrin were in general augmented at the same time as those to nicotin and adrenalin, but usually not to the same degree; in some instances they were greater, in some less. As a whole, however, they indicate that the vascular musculature itself shares to some extent in the general heightened irritability.

Similarly there was a lack of consistent parallelism between the reactions to nicotin and to adrenalin. Sometimes one, sometimes the other was greater. A comparison of the reactions to all three drugs in various cases indicates that parathyroid deficiency affects all three components of the vasomotor apparatus, — the sympathetic ganglion cells, the myoneural junctions and the musculature itself.

Keeton found that calcium injections reduce the gastric symptoms of parathyroid deficiency as they do the tetany. In case of the vasomotor symptoms this also seems to some extent to be true. A considerable experimental difficulty in determining the matter is that calcium affects the vasomotor mechanism in the normal animal causing a slower, stronger heart beat and sometimes an increased, sometimes a decreased irritability to the stimuli used.

Dog 68 showed the clearest evidence of a sedative effect of calcium. In the normal condition the animal showed augmented vasomotor irritability after 1 gram of calcium lactate given intravenously. After parathyroidectomy there was a gradual reduction of irritability for fifteen or twenty minutes after the injection of

a similar dose of calcium. After twenty minutes, however, the irritability again began to increase and was back almost to the original point at the end of a half hour.

We were able incidentally to confirm Carlson's observation that salivation is a common result of parathyroid deficiency.

No difference was observed in the results of parathyroidectomy alone and the removal of both the thyroids and parathyroids.

A possible source of error in such investigations as the foregoing is the spontaneous changes of vasomotor irritability under the conditions of our experiments. Several months' use of the technique, however, has given an appreciation of its limitations and we have no hesitancy in affirming that the increased irritability observed after parathyroid destruction is decidedly greater than the variability which occurs in normal animals. Moreover, the variability is all in one direction, whereas, in normal animals, it is of course either way, at random.

Summary and Conclusions

1. Parathyroid destruction in dogs results in a marked increase of vasomotor irritability as shown by the reactions to nicotin, epinephrin and pituitrin.

2. All components of the vasomotor mechanism, sympathetic cells, myoneural junctions and musculature, seem to be affected. The effects are of varying degree in different cases.

3. There was observed no strict parallelism between the external symptoms of parathyroid deficiency and the degree of vasomotor irritability.

4. Inconclusive evidence indicates that calcium injections in some measure restore vasomotor irritability toward normal.

5. The sympathetic system offers no exception to the general increase of irritability that results from parathyroid extirpation.

THE CONTENT OF SUGAR IN THE BLOOD UNDER COMMON LABORATORY CONDITIONS

By ERNEST LYMAN SCOTT

[*From the Department of Physiology of Columbia University, New York*]

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I. — Introduction

THE use of variations in the concentration of sugar in the blood as an indication of the response of the animal to experimental conditions offers many theoretical advantages over the use of the presence of, or variations in the amount of, sugar in the urine. This is true, first, because changes in either direction may be detected. While sugar is always present in the blood, it is ordinarily present in the urine in minimal quantities only. The urine, therefore, can ordinarily be used to show only an increase in mobilized sugar, while the blood will show either an increase or a decrease. Secondly, profound changes of concentration of sugar may occur in the blood without giving rise to a detectable glycosuria. This may be due to the short duration of the change, or the change may not be of sufficient magnitude to lead to the excretion of sugar by the kidneys or it may possibly be due to a modification of the kidneys themselves. This very sensitiveness may, however, lead to serious difficulties in the handling of the animals before and during any experiment which involves the estimation of sugar in the blood. The third and most fundamental advantage lies in the intimate relation which exists, on the one hand, between the blood and the cells which are using the sugar and, on the other, between the blood and the stores of carbohydrate.

Presumably it is the greater difficulty of technique which has deterred many investigators from using glycaemia rather than glycosuria, as the criterion of change in the organism. Too often this has detracted greatly from the value of the research. Others have recognized the more fundamental bearing of variations in the

sugar of the blood but apparently without recognizing the great delicacy of the mechanism with which they were working. The result is that in many cases well-conceived experiments are largely vitiated by inadequate or improper controls. Hirsch and Reinbach and Rolly and Opperman [1] have recently called attention to the necessity of controlling, as far as possible, every factor in experiments of this nature.

The purpose of this paper is threefold: first, to determine, if possible, a set of conditions under which the amount of sugar in the blood of one laboratory animal, the cat, will be approximately constant. Upon such conditions, when once established, experimental conditions may be superimposed with reasonable assurance that differences from the constant are due to the new factors; secondly, to study the effects of some of the common conditions to which animals are subjected before being submitted to the experimental procedures in order that those which modify the concentration of sugar in the blood may be determined; and, lastly, to study, for the same reason, a few of the experimental procedures frequently used in experiments involving the estimation of the sugar in the blood. Abundant evidence from the literature, as well as my own work, shows that the most painstaking attention to all details is demanded if trustworthy results are to be obtained.

Although, as will be seen from the above, the experiments to be reported were not primarily planned to throw light upon the problems of the mobilization and use of sugar by the organism, it is thought that some of the results found may have a deep physiological significance. An extended discussion of the theoretical or possible significance of my results would, however, be out of place at this time. The attempt is made to discover and catalogue a few of those disturbing factors which are constantly entering into our experiments, unbidden and frequently without our knowledge, and which lead us to false conclusions. Many, perhaps most, of the factors studied by me have been previously investigated for other animals and indeed some of them for the cat. It was never-

[1] References to the literature cited will be found in Section VII, arranged alphabetically according to authors. Where more than one article is cited from one author the particular articles to which reference is made is indicated by the small numbers.

theless thought desirable to correlate the results for a single animal and by a single uniform method. In part because there has been comparatively little work done upon the cat, and in part for the reasons given below, this animal was selected for the research.

Long ago Boehm and Hoffman called attention to some of the advantages of cats for laboratory work. They mentioned especially their cleanly habits, their uniform size and the fact that they had found them to be more uniformly healthy than other common animals available for estimation of the sugar of the blood. There are, however, other and perhaps more fundamental advantages. A large amount of work has been done on excised muscles — a type of experiment for which the cat seems to be particularly adapted. Notes published by Lee and by Lee and Harrold show that some of this is directly related to the use of sugar by the organism. Again, some authors, as Macleod and Pearce, have sought to avoid, by decerebration, the extended use of drugs in experiments where prolonged anesthesia is necessary. The same results may be obtained in a more physiological manner, and with less hemorrhage, by cerebral anaemia. Leonard Hill and Stewart with his co-workers have shown that the dog, because of peculiarities of the blood-supply to the brain, is not so well adapted for this procedure as is the cat. Pike has confirmed Porter's statements that cats are better adapted for experiments involving vasomotor responses than are dogs.

II. — Method of Analysis

The preparation of the animal and method of obtaining the blood will be discussed later. Only the chemical processes involved will be described here. It is not possible to estimate the sugar by any known method in the presence of protein. Many reagents have been used and many methods proposed for the removal of the protein from blood preparatory to the determination of sugar. In 1908 Michaelis and Rona[1] proposed the use of colloidal iron hydroxide for this purpose. Their method has been well received and is widely used at present. Recently, however, Lesser reports that it is not satisfactory, in the form proposed by the authors for the blood of either frogs or turtles. In the

limited use that I have made of the method I have found it fairly satisfactory but have preferred the phosphotungstic acid method described below. A method which requires but a small amount of blood for the analysis possesses many obvious advantages. Because of this the method recently described by Lewis and Benedict or the micro-chemical methods of Bang or of Michaelis will prove of great value to both the clinician and the experimentalist, provided that they give the same satisfactory results in other hands that have been reported for them by their authors. Dehn and Hartman are now publishing a series of researches in which they are developing a method for the use of picric acid as the oxidizing agent in sugar determinations. Because of the greater delicacy claimed for it the picric acid method may supplant the use of copper for this purpose. The method which I have used is very similar to the one used by Pfeffer for the removal of proteins from bacterial cultures prior to the determination of sugar. Reid and, more recently, Oppler have described methods for removing the protein from blood by this reagent.

In my own method the blood was drawn directly from the blood-vessels into a beaker weighed with sufficient 1 per cent ammonium oxalate to make the final concentration of oxalate in the blood about 0.25 per cent. The beaker was constantly shaken while the blood was being drawn. The second weighing was made at once. Any blood on the sides of the beaker was then washed down with distilled water and about 300 cc. of water added. This was done for the double purpose of preventing glycolysis, as suggested by Rona and Döblin, and of breaking down the corpuscles so that any sugar contained within them might be freed. This was suggested by the work of A. Loeb, Rona and Michaelis,[2] Rona and Takahashi and others. As soon as the laking appeared complete the solution was washed into a 500 cc. volumetric flask, which was then filled to the mark with distilled water. It was then divided into two equal portions with the aid of a 250 cc. flask, and each portion was washed into a precipitation jar. About 1.2 cc. of a freshly prepared 10 per cent solution of phosphotungstic acid was then added for each gram of blood taken. This addition was made slowly from a dropping funnel while the mixture was being stirred with a mechanical stirrer. About twenty minutes was allowed for this precipita-

tion, but, thanks to the dropping funnels and the stirrers, did not consume much time on the part of the operator. The result was a brown or chocolate colored precipitate, from which a limpid filtrate rapidly separated, that gave none of the common protein reactions. After the precipitation was complete, each portion was washed into a 500 cc. flask, which was then filled to the mark, and filtered through an ordinary filter without suction. An aliquot part of about 350 cc. was taken for analysis.

The phosphotungstic acid was removed by the addition of 25 cc. of a saturated solution of barium hydroxide. After this addition, the mixture was allowed to stand for a time at room temperature. It must not be heated at this point, nor should it be allowed to stand much longer than is necessary to complete the reaction. The completion of the reaction was determined by the addition of a few drops of the barium solution to a few cubic centimeters of the clear supernatant fluid. When the reaction was complete it was again filtered and the precipitate was well washed with water. This filtrate was rendered just acid to litmus with sulphuric acid to precipitate the excess of barium, and the barium sulphate was removed by filtration. The final filtrate was evaporated to about 50 cc. in a Jena evaporating dish, and the sugar was estimated by the "Uniform method for sugar analysis" described by Munsen and Walker. Calculations were made from the tables given in Bulletin 107, Edition of 1912, of the Bureau of Chemistry, United States Department of Agriculture. The Bulletin, in addition to the table, contains a brief description of the method.

The accurate control of the estimation of sugar in blood or other solutions containing protein is very difficult. The present method was controlled as follows. A sample of blood was prepared as described above, except that a known quantity of glucose was added to one of the two portions just before the precipitation was begun. From this time on the estimation was completed in the usual manner. Evidently the amount of sugar recovered from the portion to which the addition was made, less the amount added, should be equal to that recovered from the other portion. Reference to Table 1 will show that this was the case within the limits of error permissible for work of this type. This method presupposes that the added sugar exists in the blood in the same condition as

TABLE 1

To show Degree of Recovery of Sugar. The Blood in Experiment 6 was Divided into Four Portions: *a*, *b*, *c* and *d*. Sugar was Added to Portions *c* and *d* as Indicated. In Experiment 17 the Blood was Divided into Three Portions and Sugar Added only to *c*. Experiment 20 was Carried out just as Described in the Text.

	Experiment No. 6				Experiment No. 17			Experiment No. 20	
	a	b	c	d	a	b	c	a	b
			Glucose added	Glucose added			Glucose added		Glucose added
Gm. blood in sample	47.51	47.51	47.51	47.51	49.41	49.41	49.41	50.67	50.67
Total glucose recovered	0.0314	0.0328	0.0501	0.0497	0.0334	0.0339	0.0514	0.0358	0.0538
Less glucose added	—	—	0.0178	0.0178	—	—	0.0195	—	0.0195
Blood sugar recovered	0.0314	0.0328	0.0323	0.0319	0.0334	0.0339	0.0319	0.0358	0.0343
Blood sugar gm. per cent	0.0661	0.0690	0.0680	0.0671	0.0676	0.0686	0.0646	0.0705	0.0677
Av. of similar samples	av. a + b	0.0676	av. b + c	0.0676	av. a + b	0.0681	0.0646	—	—

that naturally present — a presumption which is by no means proved. For this reason, even though the results are satisfactory, one cannot be sure that all of the sugar has been recovered.

The degree to which results obtained by any one method are consistent, one with another, gives another means of judging of the accuracy of the method. The reader will have to be the judge of the way in which the present method responds to this test after having studied the tables submitted — especially Table 5.

Rona and Michaelis [1] have compared different methods for the removal of protein from blood and have found a variation in the amount of sugar recovered after the use of the several methods. This, they believe, is because the glucose does not all exist in the blood in simple solution. There is no present need for postulating the exact condition of the sugar, since any aggregation of the carbohydrate molecules or any combination of them with either protein or lipoid might easily interfere with the complete recovery of the dextrose by any of the methods available.

Some authors, notably Arthus, and Rosenfeld and Asher, have sought to show, by dialysis, that the sugar exists in the blood in simple solution. Consideration of the law of mass action, however, reveals the limitations of this method of attack. The equilibrium is at once destroyed by the removal of any portion of the sugar which may be in true solution. The disturbed condition will bring about a continuous dissolution of any loose combinations present so long as the removal occurs. In this way it is conceivable that a great deal of sugar may be removed by dialysis which did not originally exist in free solution.

From these considerations it follows that before the work of different authors or the results obtained by different methods may be compared, a factor of comparison must be established. That is, the amount of sugar recoverable from the same blood by each of the methods, under the same conditions, must be found, and the resulting ratios considered in making comparisons.

If it is true that the form in which the sugar is present in the blood influences the amount recoverable by the different methods, it follows that the amount recoverable by one and the same method may be expected to vary with the variation of the condition of the sugar or of any of its combinations which occurs as a result of

experimental procedures. Thus, the difficulty of interpretation of results all of which are obtained by the same method is also increased. We have at present no means of knowing that the sugar exists in the blood in the same state under different experimental conditions to which the animal has been exposed. Thus a rise or fall in recoverable sugar following any experimental change to which the animal may have been subjected, may be due to a change in the condition of the sugar in the blood with no variation in the absolute amount.

These possibilities of misinterpretation must be kept in mind in studying the following results.

III. — The Effect of Some of the Preliminary Conditions upon the Concentration of Sugar in the Blood

Under this head will come only those factors which, apart from the actual experimental conditions, interfere with the concentration of sugar.

The changes in environment undergone by an animal on entering the laboratory cannot be presumed to be without influence upon the point in question. Hence, if uniform results are to be expected, sufficient time must be allowed for all of the animals to establish themselves in equilibrium with their new surroundings. Of the many factors that might play a part in bringing about variations, two seemed especially liable to do this. These were, first, the changes in the character of the diet and feeding habits, and, secondly, the mental excitement incident to the new conditions. Time must be allowed the animals to establish themselves upon their new diet and to become accustomed to their new environment. Of the two, very probably the latter is the more productive of variations. A week seemed none too long a time to allow to the animals for this purpose, and hence was taken as the minimum limit in the usual routine. The few animals which were killed after a shorter period in the laboratory will be specially mentioned in the tables.

In Table 2 it is shown that the physical condition may be a disturbing factor. Here it is seen that the concentration of sugar may be high, as in numbers 58, 74 and 106, or be in essential

TABLE 2

To Show that the Concentration of Sugar in the Blood May be Disturbed by the Abnormal Physical Condition of the Animal

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean—Table 5		Values calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute variations	In % of stand. mean	Calculated concentration	% of var. from stand. mean	
31	—	—	126.88	—	0.075	+ 0.006	9	—	—	Abscess in jaw
58	M	2.99	107.35	35.90	0.107	+ 0.038	55	0.111	+ 61	Severe respiratory infection
60	M	2.41	75.40	31.28	0.062	− 0.007	10	0.063	− 9	Late recovery from respiratory inf.
61	M	2.92	72.91	24.93	0.068	− 0.001	1	0.064	− 7	Respiratory inf. early stages
74	M	2.50	59.43	23.77	0.086	+ 0.017	25	0.082	+ 19	Emaciated, cause unknown
95	M	2.35	79.69	33.91	0.047	− 0.022	32	0.050	− 28	Emaciated, unkempt, long standing respiratory inf.
106	M	3.12	88.80	28.46	0.101	+ 0.032	46	0.100	+ 45	Localized abscess on head

harmony with that of normal animals as shown by numbers 60 and 61; again in one case, number 95, which had apparently been running for a long time, the concentration was low. From this it is evident that one of the conditions for concordant results is the rigid exclusion of all animals which are not, so far as can be determined, in good health. While, as was shown above, a certain minimal stay in the laboratory should be allowed all animals before the sample is taken, too long a preparatory period is not desirable. The animals in general do not do as well in confinement as when free, and become especially liable to infection. For this reason, too, in experiments of long duration great care must be taken to protect the animal from all forms of infection and other influences which, aside from the purely experimental conditions, might lead to a changed physical condition.

The length of the period intervening between the last feeding and the collection of the blood may be an important factor. Bang and others have fed animals varying amounts of different carbohydrates in solution, and have followed the resulting changes in the concentration of sugar in the blood. Böe agrees with Bang that the hyperglycaemia induced in rabbits by this means has disappeared by the end of the third hour. Fischer and Wishart report a return to normal, in dogs which have ingested fifty grams of glucose in solution, by the end of the second hour. There is no doubt but that such experiments are of great value in determining the changes in glycaemia which take place under the conditions of the experiment. However, conclusions as to the conditions following an ordinary meal must be drawn with caution, since the time relations following the ingestion of protein and fat, or of these with starch, are not necessarily the same. This is true not only because of the different quantities of carbohydrate taken into the body under the different conditions, but also because of the difference in rates of absorption dependent upon the necessity for digestion in the usual meal and the interference arising from the other elements of the meal. In any case, it was thought best to allow sufficient time for any passing disturbance to disappear. With two exceptions the animals were allowed to live from sixteen to twenty-four hours after the last meal before the sample of blood was taken. In a number of cases the alimentary canals were

examined and found empty as far as the ileocoecal valve. Each of the two exceptions noted above were killed three hours after a meal, one of meat, the other of bread and meat. The one which had received meat alone, number 77, yielded 0.066 per cent sugar, which is, as will be seen by comparing with Table 5, in approximate agreement with the standard. The other cat, number 76, yielded a concentration of 0.086 per cent. This should, of course, be compared with the results shown in Table 4. When this is done it is seen that it is well within the limits of variation. Hence no significance can be attached to the variation in a single experiment from the average — 0.078 per cent — which is found for the corresponding series.

There seems to be some difference of opinion with regard to the effect of the character of the diet. Seelig finds less disturbance of the concentration of sugar in the blood of dogs given ether when the diet has consisted of bread for several days than when it has consisted largely of meat. As this point is of so much importance to the experimentalist, some attention was given to it. A diet consisting only of bread was found to be impractical for cats, so that they were given stale bread and cooked beef hearts, approximately pound for pound, together with the water in which the hearts were cooked. Even on this diet the animals did not do so well and were more subject to respiratory infection than those receiving the diet to be described later. It was not usually possible to keep them in a satisfactory condition on this diet for a longer time than two weeks. Aside from this, or perhaps because of it, a constancy of results for the quantity of sugar in the blood could not be obtained which approached that with the other diet. The results obtained are summarized in Table 4. It will be noticed that the variation between the extremes — 0.056 per cent and 0.104 per cent — is equal to 86 per cent of the smaller number and that 83 per cent vary from the average of the series by more than 10 per cent. Evidently this is not a satisfactory diet where a constant concentration of sugar is the end sought.

The other diet was cooked beef hearts with the bread omitted. This was found to be more satisfactory. While, as seen from Table 5, the extreme variation is between 0.096 per cent and 0.056 per cent and is thus almost as great as the variation of the previous

series, only 25 per cent of the animals vary from the mean by more than 10 per cent. Cf. Table 9.

Why the average for the animals allowed carbohydrate food in addition to the meat should be higher than that for those given meat alone, is a question difficult of answer. According to the ideas generally held, the character of the food is immaterial beyond the first few hours after the meal. Further, if the difference is due directly to the differences of diet, one would not look for the extreme variations which were found. There are other possibilities, however. Reference to Table 2 shows that animals with some types of infection seem to have a relatively higher content of sugar than the standard animals. As above noted, animals fed on the bread and meat diet are more prone to infection, and it is possible that in some cases incipient disease was overlooked. Again these animals were more restless and quarrelsome than those on the meat diet, and this would tend toward higher results. Rose thinks that carbohydrate feeding does not materially increase the amount of sugar in the blood of rabbits. It is, though, well to note in this connection that the rabbit is a herbivore, and as such may have better provision for handling carbohydrates than the cat, which is by nature a strict carnivore. One experiment reported by Rolly and Opperman [3] indicates that it is immaterial whether the protein given to the dog is derived from animal or vegetable sources. Jacobsen's [2] results are also of interest here.

It has long been known that the more intense emotions are a frequent cause of glycosuria. This was early spoken of by Rayer and somewhat later by Frerichs. Recently Cannon and some of his co-workers have laid especial stress upon this form of glycosuria; and have shown that for cats at least it is of purely psychological origin. Pavy [1] speaks of the necessity for "tranquillity" on the part of the animal while the sample is being drawn, and Eckhard emphasizes the fact that rabbits must not be tied in the holder for work involving glycosuria. Naunym very early reported an increased amount of sugar in the blood of animals which had been bound. Among the later writers Jacobsen,[1] Hirsch and Reinbach and Loewy and Rosenberg [1] have discussed in detail many of the difficulties in the way of the use of rabbits for experiments of this type. Presumably this difficulty lies, in large part at

least, in the nervous disposition of these animals and their proneness to excitement. Rolly and Opperman[3, 5] discard them as entirely unsuited for such work. Seelig finds no glycosuria in one dog which had been bound for two and one-half hours. Rolly and Opperman[3] also think that dogs may be safely used for such experiments, while Loewy and Rosenberg on the other hand find the concentration of sugar in the blood of both dogs and of rabbits increased by sensory stimulation, though, it is true, the increase in the dogs was not so marked.

Boehm and Hoffmann first demonstrated glycosuria in cats as a result of binding them on a holder and so called it "Fesselung Diabetes." This result has been interpreted as being due to various factors, as mental excitement, loss of heat, and muscular exertion. It has been shown in Cannon's laboratory that the first factor alone is sufficient. He therefore suggests the term "emotional glycosuria." The general fact that hyperglycaemia and frequently glycosuria follow excitement in all laboratory animals, with the possible exception of the dog, and in man is widely accepted. The only reason for adding to the already extended literature of the subject is to find to what extent the handling of an animal which is necessary for obtaining a sample of blood or in preparing it for an experiment may disturb the standard conditions. In my experiments, all animals in which excitement was evident were discarded, except as noted in the tables. A few in which excitement was evident were killed, and the results proved the necessity of Pavy's rule of complete tranquillity if consistent findings are desired. Two animals, numbers 108 and 110, were held, as if given ether by a cone, though ether was not actually given in either case. A third was placed in a bell jar for about the length of time that would have been required for etherization, had ether been given. Others were subjected to other conditions which are apt to occur in the laboratory and which produced slight excitement, as indicated by crying or otherwise. The results, with a brief description of the conditions in each case, are given in Table 3. It will be seen that in every case there is a noticeable rise in the amount of sugar contained in the blood. These results show that the animal must be, as Pavy says, tranquil, not alone at the time that the sample is drawn, but for some time before. From

TABLE 3

To Show that the Ordinary Handling to which Animals are Subjected in the Course of an Experiment may Cause a High Concentration of Sugar in the Blood.

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean-Table 5		Gm. % calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute	% of mean	Concentration	Variation in % of stand. mean	
51	F	2.60	75.57	29.06	0.102	+ 0.033	48	0.101	46	Nervous in laboratory before killing
52	M	3.50	107.65	30.76	0.078	+ 0.009	13	0.078	13	In bag 1¾ hrs. before killing, quiet
64	M	2.11	66.03	31.29	0.169	+ 0.100	145	0.170	146	Excited when brought to laboratory
71	M	3.31	61.36	18.54	0.098	+ 0.029	42	0.091	32	In bell jar 3 min. just before killing
90	M	3.45	105.63	30.62	0.133	+ 0.064	93	0.133	93	Slight excitement before killing
92	F	2.10	61.90	29.48	0.149	+ 0.080	116	0.149	116	Excited by confinement in apparatus
108	F	2.19	45.86	20.94	0.122	+ 0.053	77	0.116	68	As 64: also held rigidly 3 min. before killing
110	F	2.20	56.86	25.85	0.086	+ 0.017	25	0.084	22	Held as 108 for 8 min., previously quiet

what has been said, it will be seen that those experiments in the past in which the blood has been drawn from an artery or vein without anesthetics have a very doubtful value, since it is hardly probable that an animal will undergo such an operation and remain in perfect tranquillity. The necessary restraint is of itself sufficient to influence the results as indicated by animals number 108 and 110. Of the disturbing effect of anesthetics more will be said later. There remains then only the possibility of rapidly killing the animal with the least possible excitement and the rapid withdrawal of the sample of blood after death. This was long ago appreciated by Pavy, who killed his animals by pithing with a Bernard needle and then collected the blood from the heart or from the thoracic cavity after severing the large blood vessels. Sudden decapitation and collection of the blood from the severed neck vessels seemed to offer some advantages over Pavy's method, and was used throughout the present work. The interval during which the animal was held before decapitation seldom exceeded three seconds, while about fifteen to twenty seconds more were required for the collection of the blood. As a further precaution against excitement, an attendant, from whom the animals were accustomed to receive food, brought them to the laboratory and assisted throughout the preparation of the animal and the collection of the blood.

That the amount of blood drawn relative to the total amount in the body may affect the concentration of sugar in the sample seems to have been overlooked by previous investigators. In studying this relationship a number of standard experiments were tabulated in the order of the increasing amounts of blood drawn, when this was expressed in grams per kilo of body weight. It was then found that the respective concentrations of sugar were arranged in the reverse order: that is, the more blood drawn per kilo of body weight, the lower is its concentration of sugar. This is wholly independent of the actual amount of blood drawn, as is shown below. The phenomenon is somewhat surprising since we know that hemorrhage under certain conditions causes hyperglycaemia. The relationship of which we are speaking, however, is not to be confused with the so-called "hemorrhage hyperglycaemia" as that term is commonly used.

In the curve shown in figure 1 the data are derived from Table 5. Unfortunately the body weights of the animals used in the earlier experiments were not recorded, hence these experiments are not available for our present use. The amounts of blood drawn

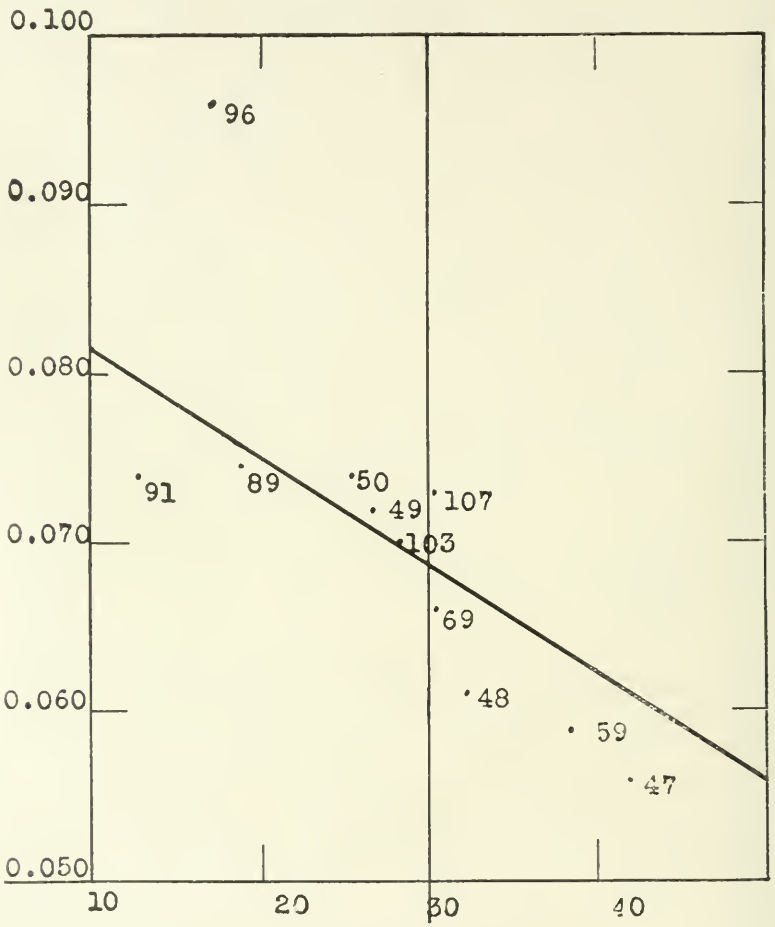

FIGURE 1

per kilo of body weight are plotted on the x-axis. The concentrations of sugar in the blood, expressed in grams per cent, were plotted on the y-axis. Provisionally the line represented by the equation $x/a + y/b = 1$ is taken as representing the relation found. In the equation x and y are variables; x represents the amounts

of blood drawn per kilo of body weight, and y the concentrations of sugar in the blood. a and b are constants whose values have not been exactly determined, but which approximate 133 and 0.084 respectively.

If this relationship between the amount of blood drawn and its concentration of sugar is constant from one animal to another, and if the above formula is its true expression, y is a constant in the equation $y = b(x' - x) / a + y'$; where a and b have the values assigned to them above. x' and y' represent respectively the amount of blood drawn per kilo of body weight and its concentration of sugar in any particular experiment. x may have any arbitrary value. Under these circumstances y represents the concentration which would have been found had x grams of blood per kilo of body weight been drawn. This calculation has been made for a number of the experiments, x being taken as equal to 30. The results, when recorded in the tables, are found in the column headed values calculated for 30 grams per kilo body weight. Since there have not been enough estimations made to warrant the assignment of exact values to a and b, the optimum position of the curve was ascertained by trial and the values were found mechanically after having plotted the results on coordinate paper as for figure 1. The results for the standard animals, calculated in the manner just described, are given in Table 5, column 10. A comparison of columns 7 and 10 of this table shows, first, that the mean for the series has not been modified; secondly, that the difference between the highest and the lowest results for the eleven animals compared is greatly reduced. Again the number of individuals which vary from the mean by more than 10 per cent is reduced from 4 to 1, or from 36 per cent to 9 per cent of the whole number of animals compared. Thus it will be seen that in general the calculated values approach still closer to a constant than do those derived directly from the analysis.

In order to test this relationship still further the blood was drawn from four animals in a series of samples in each of which the sugar was determined. The results obtained confirm those obtained by the former method and are given in Table 6. A study of this table reveals that in every case, with the exception of the third sample in experiment 95, the concentration of sugar in any sample

in the series is lower than for any sample in the same series previously drawn. These results do, however, indicate that the curve representing the relation is not a straight line, but that it falls more rapidly at first than it does later.

It was suggested that this relationship is due only to an error in analytical technique and so is of no physiological significance. For instance, it might be that a greater percentage of the sugar is recovered when only small amounts of blood are used. While it is true that such an error might lead to somewhat similar results, the

TABLE 4

THE EFFECT OF A DIET OF BREAD AND MEAT UPON THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from standard mean-table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concentration gm. %	Var. % of standard
45	F	24	2.50	93.29	37.12	0.100	+ 0.031	45	0.105	+ 52
46	M	12	2.94	101.42	34.50	0.070	+ 0.001	1	0.073	+ 6
53	M	24	2.98	83.62	28.06	0.104	+ 0.035	51	0.102	+ 48
54	F	24	2.75	79.55	28.93	0.060	− 0.009	13	0.059	− 14
55	M	24	2.50	72.66	29.06	0.056	− 0.013	19	0.056	− 19
56	M	18	2.94	65.22	22.18	0.080	+ 0.011	16	0.075	+ 9
Mean						0.078	+ 0.009	13	0.078	+ 13

criticism cannot be valid for three reasons. First, it has been found that when the sugar in unequal samples of the same blood was determined, slightly smaller concentrations were found in the smaller samples. This was probably due to some slight negative error which has a tendency to be constant. This error would be multiplied by a larger factor when the absolute amounts recovered were computed to percentages and so lead to the smaller concentrations found in the smaller samples of blood. Thus it will be seen that in so far as this error would have any tendency, it would be to

hide the relationship found rather than to simulate it. Secondly, the effect is the same whether the variation in the amount of blood per kilo of body weight is brought about by drawing different amounts of blood from animals of approximately the same weight or by drawing the same amounts of blood from animals of different weights. Compare experiments 59 with 91, and 69 with 89 in Table 5.

TABLE 5

THE CONCENTRATION OF SUGAR IN CAT'S BLOOD UNDER THE CONDITIONS WHICH WERE SELECTED AS STANDARD

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from standard mean, table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concentration gm. %	Var. % of standard
12	M	1	—	97.86	—	0.062	− 0.007	10	—	—
13	M	1	—	94.36	—	0.076	+ 0.007	10	—	—
15	—	1	—	129.73	—	0.063	− 0.006	9	—	—
16	—	1	—	60.94	—	0.063	− 0.006	9	—	—
23	—	5	—	116.33	—	0.070	+ 0.001	1	—	—
47	F	9	2.00	84.07	42.04	0.056	− 0.013	19	0.064	− 7
48	F	14	2.66	86.10	32.37	0.061	− 0.008	12	0.063	− 9
49	F	16	2.46	66.28	26.94	0.072	+ 0.003	4	0.070	+ 1
50	M	16	4.96	125.31	25.26	0.074	+ 0.005	7	0.071	+ 3
59	M	14	3.16	122.95	38.91	0.059	− 0.010	15	0.064	− 7
68	F	18	3.25	55.80	17.17	0.096	+ 0.027	39	0.088	+ 28
69	F	16	2.19	66.77	30.49	0.066	− 0.003	4	0.066	− 4
89	F	6	3.65	65.84	18.04	0.075	+ 0.006	9	0.068	− 1
91	M	25	3.05	39.56	12.97	0.074	+ 0.005	7	0.062	+ 10
103	M	8	2.23	62.61	28.08	0.070	+ 0.001	1	0.069	± 0
107	F	10	2.25	68.35	30.38	0.073	+ 0.004	6	0.073	+ 6
Mean	—	—	2.93	83.93	27.38	0.069	—	—	0.069	—

Thirdly, if the figures given in Table 6 are compared, it will be seen that the progressive decrease in concentration is entirely independent of the absolute amount of blood drawn.

No experiments have yet been made to determine the physiological significance of this decrease in the concentration of sugar when a larger proportion of the total blood is drawn at one time. The most plausible explanation which occurs to me is that it is due to the leaching of the tissue fluids into the blood vessels which occurs during severe hemorrhage. Evidence as to whether such a leaching does occur could be obtained by making simultaneous estimations of the sugar and the hemoglobin in the blood. Variations of the viscosity of the blood might also be used to throw light upon this question. Professor Burton-Opitz has found that blood drawn 25 to 30 minutes after a severe hemorrhage has a lower viscosity than that drawn before the hemorrhage. More than this, he assures me [1] that from his experience he would expect that the last of a large amount of blood drawn at one time would have a noticeably lower viscosity than would the first portions. This harmonizes directly with the theory advanced above.

Increased concentrations caused by the usual methods of withdrawal of blood have without doubt been the reason why the effect of the relative amount of hemorrhage upon the concentration of sugar in the blood has been so long overlooked. Factors are introduced by these methods which cause a greater or less discharge of the stores of glycogen, and so any small diminution in the concentrations of sugar in the blood is hidden. Pavy's method of collection should give the same results as mine, provided all the blood which has flowed from the vessels up to the time of collection is analyzed. However, I have been unable to find all of the necessary data in any of his tables. Schenck reports a very small difference between the first and second of two consecutive samples. In two experiments, the concentration of sugar in the second sample was less than in the first, and in one experiment, greater. Anderson, in two different experiments, finds almost the same concentration of sugar in each of three consecutive samples of blood. The later samples have, however, a slightly greater concentration of sugar than the earlier ones. Pavy [1] reports an increased amount of

[1] Personal communication.

sugar in the later samples of bullock's blood whether the animals were killed by the Jewish or by the pole-axe method. In none of the above were the samples so obtained that the discharge of stored glycogen would have been prevented.

Though probably sex of itself has no influence upon the concentration of sugar in the blood (cf. Bang), it is quite possible that the greater excitability of the male cats which Cannon [1] has found to exist may operate through the mechanism for emotional glycosuria to simulate such an influence. If this were true, it would be especially noticeable in experiments which involve much handling of the animal or its confinement in apparatus. While for animals kept under standard conditions I have found the mean for females slightly higher than that for males, the difference is small. Moreover, the nature of the individual variations, Tables 4 and 5, make one hesitate to attach any significance to this slight difference. It is quite possible that the results would have been otherwise for a series of animals confined in some apparatus, as a respiration chamber, for a period of time. The only evidence that I have on this is drawn from two animals which were confined in a small cage and exposed to a lowered temperature for a period of two hours. A small female cat, number 92, Table 3, resented the treatment and yielded a concentration of 0.149 per cent of sugar, while a large male, number 93, was apparently tranquil throughout the period, and yielded a result even lower than usual, 0.049 per cent. It seems to me that the stress should be laid upon the nature of the particular individual, rather than in blindly choosing animals of either sex. The sex of almost all the animals used is indicated in the tables. The results for some of the longer series are summarized in Table 7.

It is very doubtful whether there is any direct relationship between the body weight of the animal and the concentration of sugar in the blood, provided that the animals used are otherwise comparable. It must be borne in mind that variations in weight may be brought about by an abnormal physical condition, e.g., tuberculosis, and conversely these variations may be used as a means of detecting such abnormal conditions. A study of Tables 4 and 5 or of Table 8, in which the results bearing upon this point are summarized, reveals the fact that if the animals are divided

TABLE 6

THE RELATION BETWEEN THE AMOUNT OF BLOOD DRAWN PER KILO OF BODY WEIGHT AND ITS CONCENTRATION OF SUGAR

	Experiment 89 Body wt. 3.65 k.		Experiment 90 Body wt. 3.45 k.			Experiment 91 Body wt. 3.05 k.		Experiment 95 Body wt. 2.35 k.			
Order drawn	1st	2nd	1st	2nd	3rd	1st	2nd	1st	2nd	3rd	4th
Amount of blood gm.	44.89	20.95	54.2 (?)	29.29	22.04	25.47	14.09	17.55	13.93	10.43	37.79
Concentration of sugar	0.076	0.074	—	0.138	0.133	0.077	0.066	0.058	0.048	0.053	0.041
Remarks			1st sample spilled in tray, collected on weighed cotton and weighed. Not analyzed.								

TABLE 7

RELATION OF SEX TO AMOUNT OF SUGAR IN THE BLOOD

	Normal				Ether						Emotion	
	Meat		Bread and meat		Meat		Bread and meat		Street		Meat	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of indiv	6	7	4	2	6	—	4	7	4	4	4	4
Gm. per cent glucose	0.069	0.071	0.077	0.080	0.152	—	0.192	0.166	0.129	0.102	0.120	0.115

into two series, those heavier than the mean and those lighter than the mean, the average concentration of sugar in the blood of the former is slightly higher than in the latter. This difference is, however, slight, and a study of the individual variations indicate that there is no direct relationship between body weight and the concentration of sugar in the blood.

TABLE 8

SHOWING THE INDEPENDENCE OF BODY WEIGHT AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF HEALTHY CATS

	Meat fed Gm. % of sugar		Bread and meat Gm. % of sugar	
	Actual	Calculated to 30 gm.	Actual	Calculated to 30 gm.
Gm. per cent for heaviest animal in series	.074	.071	.105	.102
Gm. per cent for lightest animal in series	.056	.064	.100 .056	.104 .056
Average body wt. for series k.	2.93	2.93	2.77	2.77
Wt. of animal with most sugar	3.25	3.25	2.98	2.50
Wt. of animal with least sugar	2.00	3.05	2.50	2.50
Gm. per cent sugar for those heavier than mean	.076	.071	.085	.083
Gm. per cent sugar for those lighter than mean	.066	.068	.072	.073

IV. — THE CONTENT OF SUGAR IN THE BLOOD OF STANDARD ANIMALS

Before an interpretation of experimental work may be legitimately attempted, a standard must be fixed as a basis for comparison. It must, however, be clearly kept in mind that such a standard is no more normal than many other values which might be obtained. One of the most striking characteristics of almost all of the published tables showing the concentration of sugar in the blood is not the constancy which we have been led to expect, but a variation within rather wide limits.

One of our criteria of life is the ability of the organism to respond to changes in the environment. That is, in any environment, the organism tends to reach a condition of equilibrium and is successful in life in so far as it is successful in maintaining itself in equilibrium with its constantly changing environment. This has long been recognized for external and physical conditions, so that one would hardly say, for example, that an animal was more normal standing than walking, or asleep than awake. But internal, including chemical, readjustments must occur which are just as normal as are the more obvious physical responses. Mathews has recently spoken of the general bearing of this class of adjustments. Cannon [2] has selected the concentration of sugar in the blood of animals undergoing emotional disturbance as a type of such readjustment. He holds, and with reason, that there may be as much "purpose" in this reflex as there is in the accompanying muscular response. Indeed, the increased amount of mobilized sugar may be necessary to make the more obvious muscular response possible.

It is then hopeless to think of finding any one value which will be closely approximated by all normal animals. However this may be, the more nearly we subject the animals to a standard set of conditions before the sample of blood is obtained, the more nearly we may expect to approach a constant value. With organisms so complex as are the mammals, absolute constancy of the preliminary conditions is manifestly impracticable, and so we can hardly expect an absolutely constant value for the concentration of sugar in the blood. Again, after having established a standard value for the concentration of sugar under some one set of conditions, modification of any one or more of the factors might be expected to give a new, but none the less normal, value. Thus the addition of bread to the diet might well give a different value than meat alone (compare Tables 4 and 5).

The method of preliminary treatment which in my hands has given the most constant results, together with some of the factors which may bring about variations, has already been described. The results are given in Table 5 (compare also the values given in Table 1). Results in the other tables are to be compared with those in Table 5 as a standard, since the experiments have been

made upon animals which might otherwise have been presumed to have given similar results.

These considerations, together with the relation between the condition of the sugar in the blood and the method of analysis, make it obvious that at present only relative values for the concentration of sugar in the blood are to be expected. In order that the results of a research may be comparable, a set of preliminary conditions, which have been shown to give an approximately constant concentration of sugar, must be selected as a standard. The exact nature of these conditions will, presumably, be determined, to some extent at least, by the nature of the particular research in hand. Before the results of different researches may be properly compared, they must be reduced to similar terms. This may be done by a factor of comparison similar to the one described on page 277, but which includes the preliminary conditions as well as the method of analysis.

There seem to have been but comparatively few determinations of the sugar in cat's blood. Boehm and Hoffmann made 26 observations on blood drawn from the carotid without anesthesia. Their results are, as one would expect, high, varying between 0.11 per cent and 0.31 per cent. They may well be considered examples of emotional hyperglycaemia and should be compared with my results given in Table 3, rather than with the standard results in Table 5. Rona and Takahashi report analyses of the blood from four cats. They too drew the blood from the carotid, but under light narcosis. The concentrations which they found are quite comparable in magnitude with those of Boehm and Hoffmann, varying between 0.154 per cent and 0.355 per cent. The high concentration here is, however, probably due to the anesthetic and should be compared with results shown in Tables 10–12 rather than with my standard results. Pavy [2] gives the results of six analyses of blood taken from the heart after pithing. The values vary between 0.068 per cent and 0.1026 per cent, with a mean of 0.088 per cent. Since the type of diet and general preliminary treatment are not given, one cannot tell to what extent his results are comparable with mine, or whether they should be compared with my standard or with the results for cats fed on bread and meat which are given in Table 4. The above results are summarized in Table 9.

TABLE 9

TABLE GIVING SUMMARY OF CONCENTRATIONS OF SUGAR IN CAT'S BLOOD FOUND BY DIFFERENT OBSERVERS

Observer	Manner of collection	No. of observations	Mean concentration in %	Highest concentration	Lowest concentration	Observations which vary from average by more than 10%	
						Absolute no.	% of whole no.
Boehm and Hoffmann	From carotid no anesthesia	26	0.15	0.31	0.11	21 (?)	81
Rona and Takahashi	From carotid light narcosis	4	0.282	0.355	0.154	3	75
Pavy	From heart after pithing	6	0.088	0.103	0.068	5	83
Scott	From neck vessels after decapitation	22	0.069	0.096	0.056	4	18

V. — THE RELATION OF A FEW OF THE ORDINARY EXPERIMENTAL PROCEDURES TO THE CONCENTRATION OF SUGAR IN THE BLOOD

From what has been said it will be seen that any experiment which involves the estimation of the sugar in the blood would be valueless if the animal is subjected to pain or other form of excitement during the course of the experiment or within a few hours previous to it. It will also be noted that this is quite apart from any humanitarian considerations. Any work therefore which would otherwise involve pain must be accompanied by an anesthetic. This at once brings up the question of the effect of the anesthetic itself.

That ether, occasionally at least, causes glycosuria in patients undergoing operations has been known almost from the beginning of its use as an anesthetic. Harley and Tiegel very early demonstrated glycosuria in animals to which ether had been given. Hawk maintains that it always occurs in dogs when ether is used as an anesthetic, and his statements have been confirmed by Seelig.

Underhill publishes figures showing an increase in the concentration of sugar in the blood of two dogs which had received ether.

The fact that the administration of ether is accompanied by hyperglycaemia does not of itself preclude its use in experiments of this character. If a set of conditions — of which ether is one — can be found that meets the requirements of a standard, it would seem that the use of ether would be legitimate. In the use of ether, difficulty is at once encountered in getting the animal under the influence of the drug without introducing other disturbing factors. In Table 3 it was shown that the rigid holding of the animal necessary in the use of the cone for this purpose is productive of a significant disturbance in the concentration of sugar in the blood. Likewise, in the single case tried, a similar result was obtained when the animal was confined under a bell jar. This particular animal, however, resented the confinement. It was found that by careful selection of individuals those could be found which so far as one could tell were not disturbed by the brief restraint necessary. The bell jar has the disadvantage of offering greater danger of partial asphyxiation than does the cone, and it has been abundantly shown that asphyxia of itself is sufficient to cause hyperglycaemia (cf. Bang). It was thought that with proper precautions any danger of asphyxia could be avoided and that aside from this there were fewer objections to the use of the bell jar. Consequently in all of my experiments, where ether or chloroform was given, the animal was put in a bell jar for the initial stages. The animals were removed from the jar as soon as muscular relaxation had occurred. When the anesthetic was to be given for a longer time, this was done by means of a cone. Asphyxia was avoided either by very rapid anesthetization in a jar of fairly large volume or by the admission of air below the jar when slower anesthetization was desired.

The results for animals prepared in the standard manner are shown in Table 10. It is evident that there is no approximation to a constant. And in addition to this the animals to which ether was given for 30 minutes have a distinctly higher concentration of sugar in their blood than those to which it was administered for only three minutes or less. This indicates a cumulative effect of the ether, which would still further confuse the results of the experiment.

TABLE 10

THE AMOUNT OF SUGAR IN THE BLOOD OF CATS PREPARED IN STANDARD MANNER + ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% var. from		Values calculated. for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series	Concentration	% of var. from mean of	
											This series	Stand. series
66	M	16	3.45	.92	1.00	79.38	0.113	+ 3	+ 64	0.108	+ 1	+ 57
73 [1]	M	12	3.29	2.67	3.00	97.20	0.132	+ 20	+ 91	0.132	+ 23	+ 91
74	M	12	2.50	2.17	2.17 +	59.43	0.086	− 22	+ 25	0.082	− 23	+ 19
Mean	—	—	—	—	—	—	0.110	—	+ 59	0.107	—	+ 55
78 [2]	M	14	3.91	1.50	30	89.45	0.133	− 32	+ 93	0.128	− 34	+ 86
81	M	14	2.10	3.67	30	71.31	0.302	+ 54	+ 338	0.305	+ 56	+ 342
86	M	9	2.80	2.75	30	84.43	0.153	− 22	+ 124	0.153	− 22	+ 124
Mean	—	—	—	—	—	—	0.196	—	+ 184	0.195	—	+ 183

[1] Slight struggling when brought to laboratory.
[2] Shivering last ten minutes.

Seelig reports that ether gives much less trouble in this way with dogs which have been fed on bread for some time than with those which have been on a meat diet. Macleod also has sought to avoid the disturbing effect of ether in the same way. The results which I have obtained with cats fed on the bread and meat diet described on page 281 are shown in Table 11. A comparison of these results with those given in the preceding table shows that while there is still so much variation that they would be unsatisfactory as a basis for experimental results, they are more uniform than those obtained with the meat diet. Also the cumulative effect of the ether is not so great.

A definite relation between the ease with which the equilibrium of the mobile carbohydrates of the body is disturbed and the type of diet given the animal would be of considerable theoretical interest. Such a difference must imply a difference either in the chemical form of the carbohydrate or in the tissues in which it is stored. This theoretical interest, together with the opportunity which might be offered the experimentalist of reducing the variations to a minimum, would warrant sufficient work to establish either the existence or non-existence of such a relation. This is especially true, since Seelig's results agree with those given above in indicating the hopeful outcome of such a research.

The results given in Table 12 were obtained from animals which were to be used by a class of medical students. These animals were killed by decapitation as usual, but without special preparation. The first eight were anesthetized by ether in a bell jar in the usual manner by the students. As soon as muscular relaxation had occurred, they were removed from the jar and decapitated at once. The last five were used for demonstration purposes and had been under ether for periods varying from an hour to three or four hours, during which time the operation indicated had been done. Since these animals were primarily used for other purposes, I was unable to record the full data. The results are, however, given in the hope that they will prove of some value, indicative as they are of the results which may be expected under ordinary laboratory conditions. Chloroform does not seem to offer any advantages over ether (Harley) and has the disadvantage of a greater toxicity. A few experiments of my own, likewise, give no indication of any

TABLE 11

THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS FED ON BREAD AND MEAT AND WHICH HAVE BEEN GIVEN ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% Var. from		Values calculated for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series 0.078	Concentration	% of var. from mean	
											This series	Stand. series 0.078
63	F	11	2.49	1.00	1 +	79.99	0.172	+ 15	+ 120	0.173	+ 16	+ 122
65	F	12	2.45	1.17	1.17 +	63.42	0.135	− 9	+ 73	0.132	− 11	+ 69
67	M	17	2.12	1.67	2.67	53.30	0.170	+ 14	+ 118	0.167	+ 12	+ 114
70	F	19	2.17	3.50	3.50 +	58.82	0.166	+ 11	+ 113	0.164	+ 10	+ 110
72	F	20	1.84	3.00	4.00	52.34	0.142	− 5	+ 82	0.141	− 5	+ 81
79	F	23	2.26	2.17	2.17 +	80.55	0.111	− 25	+ 42	0.115	− 23	+ 47
Mean	—	—	—	—	—	—	0.149	—	+ 91	0.149	—	+ 91
75	M	8	2.02	3.50	24	70.93	0.213	+ 3	+ 173	0.216	+ 6	+ 177
80	M	8	3.35	2.50	30	61.22	0.215	+ 4	+ 175	0.208	+ 2	+ 167
82	F	7	2.80	3.83	67	65.30	0.155	− 25	+ 99	0.150	− 26	+ 92
83	M	7	2.25	1.50	29	57.06	0.171	− 17	+ 119	0.163	− 18	+ 115
94	F	29	2.65	4.00	34	80.48	0.280	+ 35	+ 259	0.280	+ 37	+ 259
Mean	—	—	—	—	—	—	0.207	—	+ 165	0.204	—	+ 161

TABLE 12

EFFECT OF ETHER ON CONCENTRATION OF SUGAR IN BLOOD OF CATS WHICH HAVE RECEIVED NO ESPECIAL PREPARATION

No. of Exp.	Sex	Body wt. k.	Amt. of blood gm.	Concentration sugar gm. %	Per cent of variation from mean of			Remarks
					This series	Stand. series	Bread and meat series	
37	M	—	80.05	0.106	− 8	+ 54	+ 38	
38	F	—	75.25	0.104	− 10	+ 51	+ 33	
39	M	—	97.25	0.151	+ 31	+ 119	+ 94	
40	F	—	52.60	0.094	− 18	+ 36	+ 21	
41	M	—	80.60	0.123	+ 7	+ 78	+ 58	
42	M	—	63.25	0.133	+ 16	+ 93	+ 71	
43	F	—	49.45	0.126	+ 10	+ 83	+ 62	
44	F	—	62.05	0.084	− 27	+ 22	+ 8	
Mean	—	—	—	0.115	—	+ 67	+ 47	
25[1]	—	—	91.25	0.129	− 40	+ 87	+ 65	Pleural puncture, treacheotomy
57[1]	M	3.00	80.89	0.134	− 38	+ 94	+ 72	Decortication
62[1]	M	3.25	55.66	0.239	+ 11	+ 246	+ 206	Decortication less ether than no. 57
84[1]	M	2.80	79.92	0.298	+ 39	+ 332	+ 282	
85[1]	M	2.75	72.80	0.274	+ 27	+ 300	+ 251	Respiration stopped under ether
Mean	—	—	—	0.215	—	+ 212	+ 176	

[1] These animals were used for class demonstration and were under ether for at least one hour and in addition were subjected to the operations indicated.

advantage to be derived from its use, since the results are essentially similar to those obtained by the use of ether (Table 13).

Because of its stimulating action on the cat, no one would think of making use of morphine in drawing blood from this particular animal. It is, however, of interest to note that Luzzatto finds glycosuria following the use of morphine in rabbits. This finding is confirmed by Araki, who also reports similar results for

TABLE 13

THE RELATION BETWEEN CHLOROFORM AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF ANIMALS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Body wt. k.	Time in min. from application of chloroform to		Amt. of blood drawn gm.	Concentration sugar gm. %	% var. from mean		Remarks
			Musc. relaxation	Death			This series	Stand. series	
87	M	3.79	5.0	5.17	78.05	0.105	− 9	+ 52	Unusually quiet before and during anesthetization
88	M	3.50	2.0	2.67	67.94	0.098	− 15	+ 42	
97	F	2.75	1.5	1.57	62.96	0.142	+ 23	+ 105	
Mean	—	—	—	—	—	0.115	—	+ 67	

dogs, though he found no sugar in the urine of frogs after morphine. On the other hand, Hirsch and Reinbach think that morphine is without effect on the concentration of sugar in the blood of rabbits. Jacobsen found an undoubted increase in the amount of sugar in the blood of rabbits to which sufficient chloral had been given to produce narcosis.

Some investigators have collected blood from one of the large vessels under the local anesthesia produced by cocaine (Fisher and Wishart). For some types of experiment, such a method is particularly desirable, provided the equilibrium of the mobile sugar is not disturbed by the drug in such a manner that the proper allowances cannot be made. Araki found lactic acid in the urine of frogs and of rabbits after the injection of cocaine. One of the four rabbits injected also secreted sugar with the urine. In the present work four cats were injected beneath the skin of the back with large doses of cocain hydrochloride dissolved in N/8 sodium chloride solution. These animals were all killed in the early stages of the apparent reaction to the drug. (See Table 14 for details.) With one exception each of the concentrations of sugar found was well below the standard concentration. The mean concentration for the series is 86 per cent of the standard mean. Any attempt to explain this finding woulP be premature, since a longer series

TABLE 14

THE EFFECT OF COCAINE UPON THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Days on diet	Body wt. k.	Amt. cocaine injected gm.	Time between injection and death in min.	Amt. of blood drawn gm.	Concentration sugar in blood gm. %	% Var. from mean of		Remarks
								This series	Stand. series	
98	F	2	2.58	0.07	8	68.79	0.063	+ 7	− 9	Killed in 1st stages of stimulation by drug
100 [1]	M	3	2.85	0.05	10	87.40	0.055	− 7	− 20	No other excitement
101	F	6	2.37	0.07	6	57.38	0.048	− 19	− 30	As No. 98
102	M	8	2.68	0.07	6	77.85	0.070	+ 19	+ 1	Killed after symptoms of the drug were marked
Mean	—	—	—	—	—	—	0.059	—	− 14	

[1] Only slight symptoms of drug at time of death.

might yield results which would essentially modify the situation. Also too little is known at present of the other factors of metabolism during intoxication by cocaine to warrant such an attempt.

In following the progress of an experiment it is frequently desirable to determine the changes in the concentration of the sugar in the blood at frequent intervals. Unfortunately there is, however, a very serious objection to this procedure. Claude Bernard found that the concentration of sugar is increased by a previous hemorrhage, and his finding has been repeatedly confirmed. Recently fairly exhaustive studies have been made by several authors. Among others Anderson, Jacobsen, Rose and Schenck have studied this effect in rabbits. Anderson found an increased concentration of sugar five minutes after the hemorrhage, but did not determine whether it was present after a still shorter interval. The consensus of opinion is that the concentration reaches its maximum about thirty minutes after the hemorrhage and that it remains high from three to four hours.

Undoubtedly emotional disturbances have frequently contributed a large share to the so-called hemorrhage hyperglycaemia. However this may be, there is no doubt that quite apart from any disturbance due to emotion or to anesthetics, hemorrhage does introduce a modification for which proper controls must be made. Some authors have sought to avoid the introduction of the factor of hemorrhage by the use of a quantity of blood so small that it might be considered as negligible, but this so greatly increases the probable error from analytical technique that the method has a questionable value, at least for most methods of analysis. Furthermore the objection to repeated handling of the animal and the consequent excitement are not met by the change in analytical method, and demand exceptional skill on the part of the experimenter. The literature covering this subject is so ample and, taken as a whole, so conclusive that it was not thought necessary to add to it.

Changes in the concentration of the sugar in the blood may be used as a measure of the effect of a substance which has been injected into the animal. In such experiments it is usually presumed that the effect of the injection aside from the drug is nil. My own experiments are too few in number to allow of general conclusions.

But in harmony with the rest of my work they indicate the necessity of complete control of all factors in the experiment. Long ago Bock and Hoffmann showed that large amounts of salt solution caused glycosuria when injected intravenously. But while drugs are frequently dissolved in a solution of sodium chloride for injection, the effect of the salt solution is essentially different from that obtained by Bock and Hoffmann, since usually very much smaller amounts are injected. In my own experiments with cocaine certainly no factor was introduced which increased the concentration of sugar enough to conceal the results due to the cocaine alone, with the possible exception of one animal. (See Table 14.) Especial care was taken in making these injections of cocaine to avoid exciting the animal. The same care was exercised in an animal which was injected with 5 cc. of M 8 sodium chloride. This cat was killed five hours later, and the blood yielded a concentration of sugar of 0.0697 per cent, a result almost exactly the same as the standard. Another animal injected through an opening in a small box in which it was confined with the same amount of sodium chloride solution became much excited. This animal, after the lapse of a similar interval, yielded a concentration of 0.098 per cent — a much higher result than the standard.

Again, many experiments of this nature involve the confinement of the animal within some form of apparatus. The exact results obtained in animal calorimetry are ample evidence of the availability of this type of research. On the other hand, great care is necessary to avoid exciting the animal. This is illustrated in the two animals exposed to cold as described on page 291. While they were exposed to similar external conditions, one became very restless and yielded a concentration of 0.149 per cent of sugar; the other remained exceptionally quiet and yielded a concentration of only 0.049 per cent. The nature of this experiment, together with the results, would suggest the possibility that the unpleasant conditions involved constitute at least one of the factors leading to the mobilization of the sugar in Lusk's method of ridding the body of glycogen by shivering.

In another experiment four cats were confined in a respiratory chamber and subjected to a temperature of about 32° C. and a relative humidity of about 88 per cent. The results are shown in

Table 15. Animals were selected for these experiments which might be expected to remain quiet throughout the experiment. No. 112 was the only one which proved disappointing in this regard. The results show an exceptionally low average for the concentration of sugar, and this individual is the only one of the four which reached the level of the standard cats.

TABLE 15

THE EFFECT OF THE CONFINEMENT OF CATS IN A WARM, MOIST CHAMBER UPON THE CONCENTRATION OF SUGAR IN THE BLOOD

No. of Exp.	Sex	Body wt. k.	Amt. of blood drawn gm.	Hrs. in chamber	Temp. mean C.	Rel. humidity mean	Concentration sugar in blood gm. %	% of var. from stand. mean	Cal. for 30 gm. blood per k. body wt.	
									Concentration sugar gm. %	% var. from stand. average
111	F	3.65	83.38	6	30.6	.83	0.053	– 23	0.049	– 29
112[1]	F	1.62	55.57	6	30.7	.89	0.064	– 7	0.067	– 3
113	F	3.78	69.87	6	33.1	.90	0.065	– 6	0.058	– 16
114	F	2.90	65.12	6	32.9	.90	0.059	– 14	0.054	– 22
Mean	—	—	—	—	31.8	.88	0.060	– 13	0.057	– 16

[1] Excited when removed from the chamber.

There seems then to be no reason for attributing changes in the concentration of the sugar in the blood following the injection of small amounts of salt, or the confinement of the animal in apparatus to these conditions of themselves. Excitement induced by these conditions may, however, give rise to high concentrations, even five hours after the time of irritation.

VI. — SUMMARY AND CONCLUSIONS

1. Glycaemia offers a more satisfactory indication of the condition of mobile sugar than does glycosuria; first, because either an increase or a decrease in the amount of sugar may be demonstrated, while normal urine can show only an increase; secondly,

because profound changes in glycaemia may occur in response to conditions which do not produce glycosuria; thirdly, the blood is in much more direct relation to the living cells than is the urine.

2. The concentration of sugar in the blood as estimated by different methods varies. This may be due, in part at least, to the form in which the sugar is present in the blood. It follows that results obtained by different methods of analysis cannot properly be compared until they have been reduced to common terms. Also the possibility is introduced of an apparent variation in concentration of sugar, even when the method of estimation is constant, which is due to a change in the form in which the sugar is present rather than to a change in the actual amount of sugar present.

3. If consistent results are to be expected, the animals must be uniformly healthy, and must be killed without pain or excitement. Sex or weight, apart from correlated conditions, are probably without special influence upon the concentration of sugar in the blood.

4. The normal concentration of sugar may very probably vary with the varying environment of the animal or with changes in its physical state. However, if the environment is uniform and if the animals are killed while in the same physiological condition, constant results should be expected. Practically such an ideal result is not possible, but has been approached with some success.

5. The concentration of sugar in the blood decreases as the amount of blood drawn per kilo of body weight of the animal increases. So far sufficient data have not been obtained to establish the mathematical expression for this relation.

6. When ether or chloroform was administered, the concentration of sugar was increased considerably and varied between rather wide limits, whether the diet consisted of meat alone, or of bread and meat, the latter diet giving somewhat smaller variations than the former. After either diet there was a greater concentration after the drug had been administered for thirty minutes than after it had been administered for three minutes or less.

7. The concentration of sugar in the blood after subcutaneous injection of cocaine is more constant than that found after inhalation of ether or chloroform and is lower than that found in animals similarly treated but to which cocaine has not been given.

8. It may be shown from the literature that hyperglycaemia

follows hemorrhage. From this it follows that caution must be exercised in drawing conclusions from experiments which involve the analysis of successive samples of blood.

9. The excitement which is apt to attend hypodermic injections or confinement in apparatus may lead to high results and consequently to false conclusions, With care, however, such effects may be avoided so that this type of experiment is permissible.

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THE EFFECT OF CALCIUM AND PROTEIN FED PREGNANT SWINE UPON THE SIZE, VIGOR, BONE, COAT AND CONDITION OF THE OFFSPRING[1]

By JOHN M. EVVARD,[2] ARTHUR W. DOX,[3] and S. C. GUERNSEY[3]

[*Animal Husbandry and Chemical Sections, Iowa Experiment Station*]

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TO determine the effect of adding calcium and protein to a corn ration fed the pregnant gilt upon the relative size and vigor of the offspring, a series of experiments has been conducted at this Station. The results obtained during the year 1912–13 will be discussed briefly.

The gilts under observation were divided into three lots of ten each. Lot I received whole corn grain (shelled) only, 1279.13 grams (reduced to 14 per cent moisture basis) per head daily; Lot II whole corn grain (shelled) the same amount as Lot I, plus calcium allowed in the form of chloride and carbonate (equivalent to approximately $2\frac{1}{2}$ grams of calcium daily); and Lot III corn grain (shelled) the same in amount as Lot I plus protein fed as black albumen to the extent of 136.08 grams of the blood product per head daily. This black albumen analyzed 88.24[4] per cent

[1] Written April 1, 1914. Preliminary Report.

[2] Animal Husbandry Section.

[3] Chemical Section.

[4] The black albumen and corn as analyzed contained in a hundred grams. We used the black albumen because it was the best and purest form of commercial protein on the market. We preferred the blood derivative to the wheat gluten because of the much greater likelihood of its containing the complete series of amino acids.

The blood albumen runs higher than corn only in protein and ash. Fortunately the ash constituents of the corn exceed in potassium, magnesium, and phosphorus. The blood ash excels in sodium, chlorine, calcium and sulphur, but the possible influence of the first two, sodium and chlorine, is

protein and contained very little of the mineral elements, being especially low in calcium. All gilts received equal quantity of sodium chloride daily, namely 7.26 grams per head. The daily gains of the three groups were as follows: Lot I, 107.95; Lot II, 154.68; Lot III, 237.23 grams.

The number of pigs farrowed per sow from these three lots was, respectively: Lot I, average 7.88; Lot II, 7.30; and Lot III, 8.22. Here we notice, as in our previous experiments, that the protein added to the corn ration during the breeding season influences favorably the number of young.

The weight of the total litters, as well as that of the individual pigs, shows clearly the influence of calcium and protein respectively upon the developing fetus. The table presented on page 314 gives the number in litter, litter weight, and average weight per pig. The basis is grams.

negligible because we purposely fed a sufficiency of sodium chloride, the same to all lots. The calcium difference is so small as to be almost negligible. The results presented, wherein Lots I and II are contrasted, show plainly that calcium has some influence, and we must make some allowance for the

	Albumen	Corn
Protein	88.24	9.81
Ether extract	1.30	2.64
Ash (total)	3.26	1.42
Nitrogen free extract	none	74.83
Crude fibre	none	2.38
Moisture	7.20	8.92
Calcium	.03	.01
Phosphorus	.19	.61

extremely small but nevertheless constant difference. As regards the sulphur difference we attribute to it considerable possible influence, — but inasmuch as sulphur is to the protein much as "the tail is to the entire hide" we must charge the effects produced to the protein of the blood albumen. Summarizing, therefore, we find that the results secured by supplementing corn with black albumen are theoretically due almost entirely to its protein content.

Weight of Offspring

Lot no.	No. in litter	Litter weight grams	Average per pig [1] grams
I	7.88	6454.62	821.00
II	7.30	6695.02	916.26
III	8.22	7838.08	952.54

[1] On basis of all pigs farrowed.

The litter of the lightest weight comes from the group receiving "corn alone," whereas the heaviest is to be found where corn was supplemented with protein as in Lot III. Here we note a litter difference of 1383.46 grams in favor of the protein supplemented corn ration. The protein increased the weight of litter practically 29 per cent. The effect of the complex protein in black albumen is much more marked than that of the simple calcium fed as chloride and carbonate. The important deductions are such as to emphasize the necessity of both of these constituents, namely calcium and protein, in feeding corn to bred swine; the addition of either one of these resulting in heavier litters and larger average pigs.

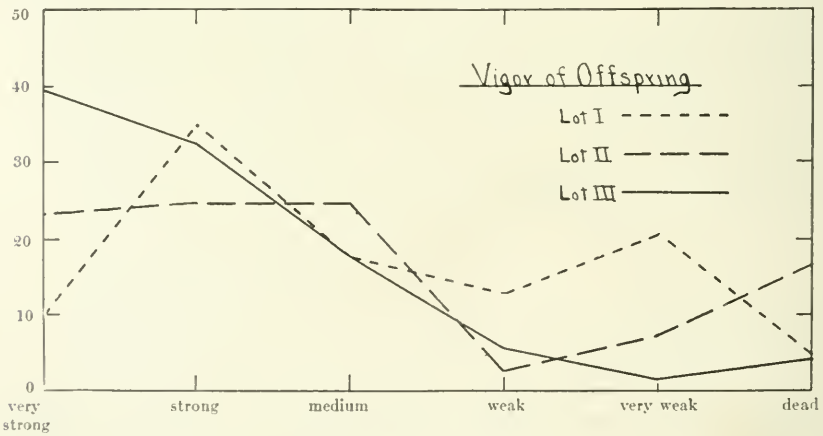

Chart 1

The vigor of the offspring was markedly affected by the ration. We have the following distribution of the pigs in the three lots according to their relative strength:

VIGOR OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Very strong	Strong	Medium	Weak	Very weak	Dead
I	9.52	34.92	17.46	12.71	20.63	4.76
II	23.29	24.66	24.66	2.74	8.22	16.44
III	39.19	32.43	17.57	5.41	1.35	4.05

Most assuredly the addition of protein affected profoundly the vigor and stamina of the offspring. (See chart I for distribution.) The addition of calcium was not without its effects. The protein, however, seems to be the more important constituent in balancing up the corn when compared to calcium.

The size of bone was likewise affected. When calcium and protein were added to the ration the bones were larger. This was determined by measuring the front and hind shins. The measurements are presented in centimeters:

SIZE OF BONE CIRCUMFERENCE

	Front Shin	Hind Shin
Lot I	4.60	4.36
Lot II	4.88	4.67
Lot III	4.81	4.56

Peculiarly enough, where calcium was added to corn the size of bone was somewhat greater [1] than where the protein was added. Now this may be due in part to the fact that the Lot III farrowed

[1] Cf. Hart, Steenbock, and Fuller, Research Bulletin 30, Wisconsin Experiment Station. "High calcium rations, as compared with low calcium rations, had no effect whatever during a single gestation period on the size or calcium content of the skeleton of the fetus. The skeleton is not increased in any dimension by a wide variation in the amount of calcium fed the mother." According to these investigations the ration considered as a "low calcium" one is a much higher carrier of calcium than the basal ration of corn used in the Iowa experiments.

a greater average number of pigs per litter which would have a tendency, other things being equal, to decrease their relative size.

One is not surprised particularly to find that both calcium and protein had considerable effect upon the size, vigor, and bone of the offspring, but the fact that the coat is likewise markedly affected is somewhat surprising. To determine the influence of the addition of the constituents above mentioned upon the quantity of coat produced in the offspring, observation being made at farrowing time, the relative coat covering upon all of the new-born pigs was carefully recorded. The table on "Coat Quantity of Offspring"

COAT QUANTITY OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Very heavy	Heavy	Medium	Light	Very light	Absent
I	3.23	29.03	41.94	24.19	1.61	none
II	8.33	34.72	40.28	9.72	6.94	none
III	21.62	40.54	32.43	5.41	none	none

gives the number of pigs out of every hundred born showing the Very Heavy, Heavy, Medium, Light, Very Light and Absent coats. A chart showing the coat quantity distribution is here given.

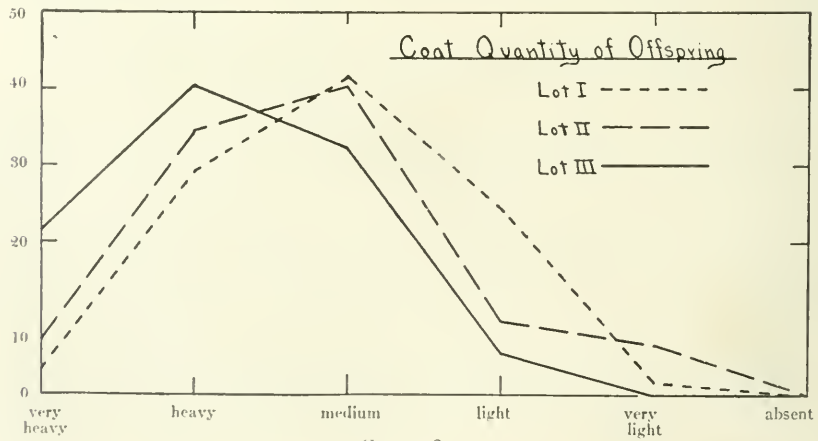

CHART 2

The calcium addition was somewhat effectual in that the coats produced from this lot were a bit heavier. The difference between Lots I and II is, however, very slight. Marked effects are shown from the protein addition where the number having very heavy coats was increased from 3.23 to 21.62 or practically seven times as many possessing the Very Heaviest. Densest coats where protein is allowed as compared to where it was not. Dropping down to the next coat quantity, namely Heavy, we find 29.03 in the check lot as compared to 40.54 where the protein was added, or more than 40 per cent difference. The Very Light coated pigs are conspicuous in Lot III for their absence, thus further demonstrating the effects of protein additions in increasing the amount of hair covering.

That the coat of swine should vary according to the feed given is common experience. Just one month after these young gilts were placed on the experimental rations a marked contrast in the quantity and color of the coats was evident. The coats of hair in the order of their length and density are from Lots III, II, I, with II and I fairly close and III easily first. In color we have the same order, III, II, I, with III much the darkest. It is significant that the coat quantity and color should be affected by the ration, — it is still more suggestive that the coats of the new-born should correspond somewhat with those of the dams from which they were farrowed.

What is the explanation of this difference? We know that keratin, a simple protein of albuminoid nature, is the chief constituent of hair. We find keratin in the epidermis, wool, nails, hoofs, horns, feathers, and so on. Keratin is peculiar in that it has a high sulphur content, the sulphur being present largely in the form of the complex amino acid cystine.

The keratin of human hair runs as high in cystine as 13 to 14½ per cent.[1] No other protein runs so high in cystine as the keratin of human hair. Swine hair or bristles contain about 7.2 per cent cystine. Most assuredly hair cannot be built unless the constituents of cystine are present in the feed, hence it is reasonable to suppose that if said sulphur compound, namely the amino acid cystine, is absent from the feed, the development of hair may be

[1] Buchtala: Z. Physiol. Chem., Volume 52, page 474, 1907.

retarded. In corn we find approximately .171[1] parts of sulphur in 100 parts of dry matter, whereas in black albumen we have .820[1] parts or almost five times as much, furthermore it has been shown that of the sulphur present in zein, the protein that comprises 58 per cent of the proteins of corn, only 35 per cent[2] is present as cystine. On the other hand a large proportion of the sulphur found in black albumen is supposedly present as cystine, hence it is not unreasonable to assume that the addition of black albumen furnishes the cystine, the basal constituent of hair growth.

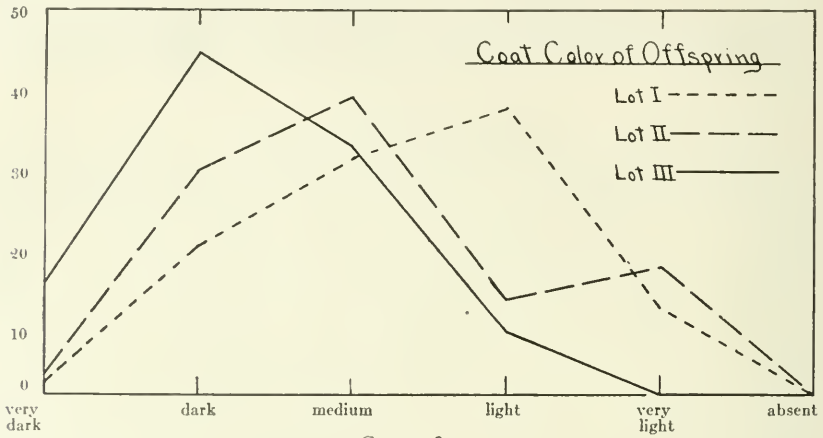

CHART 3

The coat color of the offspring differs, depending upon the dietetic treatment accorded the pregnant dam. The relative effects of the supplements upon the color is illustrated quite clearly in the table on the opposite page showing the number of pigs out of a hundred farrowed classified as Very Dark, Dark, Medium, Very Light, and Absent coat colors:

Again we see the effects of the added black albumen in that it increases the general coat color of the offspring. The chart showing color distribution plainly demonstrates the differences. The hogs which we used were Duroc Jerseys, having red coats. The coats designated as "Very Light" refer to those of little color as compared to the "Very Dark" coats which were of a bright cherry red.

[1] FORBES: Bulletin No. 255, page 225, January, 1913, Ohio Experiment Station.

[2] BUCHTALA: Z. Physiol. Chem., Volume 52, page 474, 1907.

Coat Color of Offspring
(On basis of 100 pigs farrowed)

Lot no.	Very dark	Dark	Medium	Light	Very light	Absent
I	1.61	19.35	30.65	37.10	11.29	none
II	2.78	29.17	38.89	12.50	16.67	none
III	14.86	44.59	32.43	8.11	none	none

The calcium did not seem to affect the coat very much, although it shows a minor influence. The black albumen with its high protein, and possibly its specific cystine content, seems to be the causative agent in the production of highly colored coats. It is to be understood that the coat color markings are affected by the amount of coat present, depending upon whether the hairs are densely studded on the surface of the body as well as the length of the hair, and furthermore on the inherent color of the hair itself. As far as superficial observation goes, without entering into the details of microscopic technical examinations, we would give it as our judgment that the coats were not only denser and longer but that the hairs themselves seemed to show a greater amount of pigment when corn was supplemented with the black albumen protein as compared to corn fed alone.

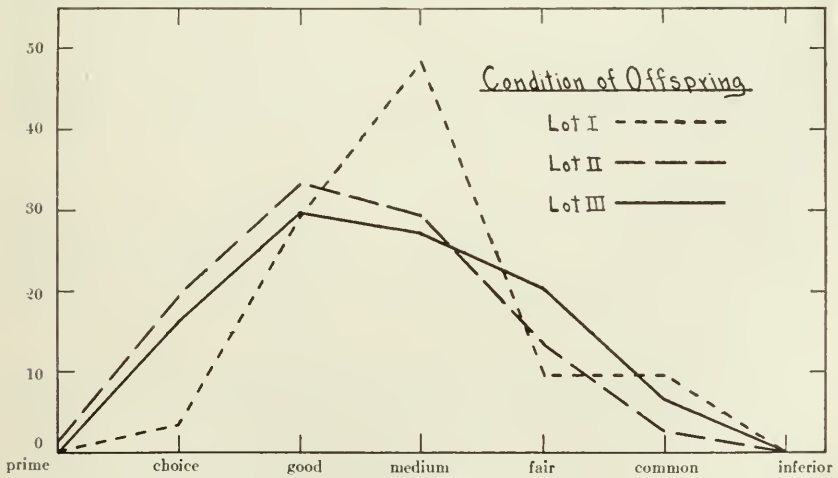

Chart 4

The condition or degree of fatness of the new-born pigs is somewhat dependent upon the feed allowed the dam during the period of gestation. To demonstrate the effect of specific supplements to corn upon the relative condition of the offspring we append herewith table showing the degree of fatness of the various new-born pigs farrowed in the three lots:

Condition of Offspring
(On basis of 100 pigs farrowed)

Lot no.	Prime	Choice	Good	Medium	Fair	Common	Inferior
I	none	3.23	29.03	48.39	9.68	9.68	none
II	1.39	19.44	33.33	29.17	13.89	2.78	none
III	none	16.22	29.73	27.03	20.27	6.76	none

The condition or fatness of the new-born pigs was determined by sight and touch observations. Each pig was handled so that a fairly accurate estimate could be made of the fatty covering, special emphasis being placed upon the superficial layers over the ribs and back. Both the calcium and protein supplements to corn resulted in fatter offspring. The protein in this case had less effect than the calcium. Our estimates of the condition of the dams producing these pigs placed the lots in order of fattest first, thus: III, II, I, whereas the condition of the pigs farrowed of said dams, placing the fattest first, is II, III, I. In other words the condition of the resulting offspring does not compare as closely with that of the mother as does the coat character. There are obvious fundamental reasons for this.

To recapitulate so as to put the foregoing vigor, coat, and condition story on a comparative and more easily interpretable basis there is summarized the relative effects of the specific feed constituents in a grouped combination table chart. (See Chart 5 on opposite page.)

The perpendicular columns denote the average on the assumption of the highest marking being perfect, or 100. The average is computed by placing a value on the various markings given the individual pigs, — thus for vigor the Very Strong pig is credited

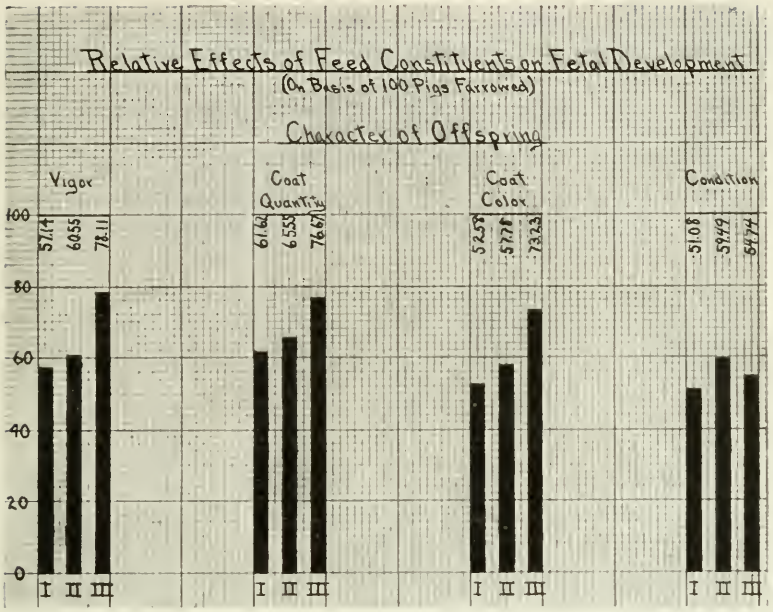

Chart 5

with 100, Strong 80, Medium 60, Weak 40, Very Weak 20, and Dead 0. The Dead with 0 and the Very Strong with the 100 credit makes the range from Absent vigor, the lowest, to Very Strong vigor, the highest marking. The total vigor credits are added and the average taken with results in Lots I, II and III, respectively, of 57.14, 60.55, and 78.11. These values may be regarded as percentages of the maximum vigor marking, and so on.

The same general scheme was followed out in determining the average "Coat Quantity," "Coat Color," and "Condition." The gradations considered are identical with those on the tables and charts previously presented.

Withal, this method gives us a tangible, definite, interpretable average valuation quite in accord with the facts.

Uniformly the supplemental calcium and protein, respectively, produced improvement in specific characters of the offspring. Manifestly the influence of the complex nitrogenous organic constituent protein is more marked than that of the more simple inorganic calcium (chloride and carbonate).

The relative influence of calcium and protein is more clearly appreciated on examination of the following table: —

Comparative Influences of Calcium and Protein Fed the Pregnant Dam on Developing Fetus

Character of offspring	Percentage increase over corn alone attributable to	
	Calcium	Protein
Vigor	5.97	35.00
Coat quantity	6.38	24.42
Coat color	9.89	38.04
Condition	16.46	7.17

Perhaps the direct comparison of protein to calcium effectiveness would make the relation of these two constituents clearer.

The increase of the Protein-Corn-Lot III over the Calcium-Corn-Lot II shows for

Vigor	29.00 per cent
Coat quantity	16.96 " "
Coat color	26.74 " "
Condition	7.90 " "

The protein is more effective than the calcium in the promotion of vigor, production of coat quantity and color, but less so in augmenting the condition.

Evidently the protein is the more efficacious when it comes to the production of those qualities which make for stamina and hardiness. The vigor and coat quantity are relatively more important in lessening the mortality of the suckling than is the degree of fatness. If the new-born be strong, healthy, and well-coated, even though he come into existence under adverse conditions he is much better adapted to live in the environment he finds than if he lacks vigor and coat but possesses a high degree of fatness. The strong, warmly coated pig will soon fatten on his mother's milk, hence the condition comes quickly. Not so however with

the strength and coat; lost vitality and scant hair covering are replaced with comparative slowness.

It is vital to early development that the new-born pigs be vigorous, otherwise they will be compelled to suckle the teats discarded by the more active individuals in the litter. "That pig which suckles the hind teat" is at a disadvantage, but this is the consequence, usually, of being farrowed as the weakly member of the litter.

The protein in corn has been demonstrated to be deficient to a considerable extent in some of the essential amino acids. This is especially true of the zein which comprises practically 58 per cent of the corn proteins, since zein does not contain in its amino acid make-up tryptophane, lysine, and glycine. Fortunately for corn the glutelin which furnishes most of the remaining protein is quite complete in its amino acid constitution. However, the marked preponderance of zein in corn lessens greatly the general efficiency of the protein in toto. The tryptophane [1] is probably the limiting amino acid, hence it is reasonable to assume that the addition to the corn ration of a protein rich in tryptophane would show marked results. We are led to believe from the work already done on the amino acid content of blood and its derivatives that the blood albumen used as the source of protein in our work carries the deficient tryptophane. Perhaps the possible deficiency of cystine in corn as heretofore noted may be a factor, the absence of which contributes to the general inefficiency of the corn proteins. The balancing therefore of the protein present in corn by making it more complete, as well as an increase in the entire amount fed, should be a double reason for the greater efficiency observed.

We had some difficulty in the administration of our calcium. We first started out with calcium chloride but found that where it accompanied protein, given in the form of black albumen, difficulty was experienced in that the mixture seemed to have antagonistic relations. We have supposed that this may possibly be due to acidosis caused by the liberation of the chlorine portion of the calcium chloride molecule, thus freeing hydrochloric acid. Along with a high protein ration the demand for calcium would necessarily

[1] Osborne: "The Nutritive Value of the Proteins of Maize" — Science N. S. 1913, xxxvii, page 185.

be greater than where no extra protein was fed, hence we should expect a greater demand for calcium under these conditions with a correspondingly greater liberation of chlorine which would induce acidosis. This acidosis would theoretically be largely done away with by the feeding of a pure calcium limestone such as calcium carbonate. We found when calcium chloride was replaced with calcium carbonate, feeding same between meals, that the ill effects heretofore noted were largely eliminated. Observation and trial showed however that calcium carbonate should not be mixed with the feeds as allowed but that it should be fed preferably between meals. We are further investigating this problem in order to demonstrate the best way to feed the calcium.

It is reasonable to suppose that calcium will give results when added to the corn ration as corn is especially lacking in this important mineral element which comprises 40 per cent of the dry ash of bone. Calcium furnishes 70 per cent of the basal elements of bone, the remaining $29\frac{1}{2}$ per cent being supplied by phosphorus and $\frac{1}{2}$ per cent by magnesium. In the normal human body there is just about two-thirds as much calcium as nitrogen, that fundamental element of protein concerning which we hear so much and upon which a maximum of emphasis is invariably placed by feeding experts and dieticians. It is not to be gainsaid that the lack of protein is the more conspicuous deficiency in ordinary grain diets, — but nevertheless the calcium deserves among the mineral nutrients considerably more attention than is now accorded.

Much of a conflicting nature has been said by obstetricians, dieticians, and the laity concerning the effect of different food constituents upon the development of the embryo and fetus.

The experience at the Iowa Station, involving over 2000 newborn pigs, shows beyond all reasonable doubt that the addition of meat to the ordinary cereal diet of pregnant swine has very marked influence upon the size and vigor of the new-born.

All work heretofore done at the Iowa Station has plainly indicated that the addition of mineral elements as well as protein to the ration had its marked effects upon the development of the young in utero. We are led to believe that any feed, including water, added to or subtracted from the ration of pregnant swine which will tend to promote or discourage growth, thrift, and vigor

of the dam will within reasonable limits have its effect upon the developing fetus.

Summary

1. Corn maize is markedly deficient in calcium and quite low in protein, the major part of which lacks certain important amino acids.

2. The addition of calcium (allowed as chloride and carbonate) to a fixed basal ration of corn and sodium chloride with pregnant gilts resulted in new-born pigs having greater size, more vigor, bigger bone, increased coat quantity, better coat color, and higher condition.

3. The addition of a high protein feed (Black blood albumen) resulted in the new-born pigs having greater size, more vigor, bigger bone, increased coat quantity, better coat color and higher condition.

4. The influence of the complex organic protein is more marked generally than that of the more simple inorganic calcium.

5. The use of chloride as the source of calcium was not as satisfactory as the carbonate in a high protein ration, presumably because of the undesirable liberation of chlorine causing a possible condition of acidosis.

6. The ration fed the pregnant mother affects in a marked degree the general development of the fetus.

THE INFLUENCE OF ADRENALIN ON RESPIRATION

By L. B. NICE, JOHN L. ROCK and R. O. COURTRIGHT

[*From the Laboratory of Physiology in the University of Oklahoma*]

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IT has been shown by several investigators that the introduction of small doses of adrenalin into the circulatory system causes a fall in blood pressure.[1] Large doses on the other hand cause a rise in blood pressure.[2] As to the effects of adrenalin on the respiratory system, comparatively little work has been done. Oliver and Schäfer[3] found that extracts of adrenal glands cause a shallowness in the depth of respiration. Langley confirmed this result, stating that the respiratory mechanism responds readily to the first injection, but that succeeding injections bring forth insignificant responses.[4] Similar results were obtained by Badano,[5] and later by Boruttan[6] and Kahn.[7] All of these experimenters used large doses of adrenalin.

The purpose of the present study was to investigate the effects of small (physiological) doses of adrenalin on the respiratory mechanism. In order to ensure that the doses were being given accurately and uniformly, the effects on the blood pressure were recorded, as well as those on respiration.

[1] Moore and Purington: Archiv für die gesammte physiologie, 1900, lxxxi, p. 483; Elliott: Journal of physiology, 1905, xxxii, p. 411; Dale: Journal of physiology, 1905, xxxii, p. 59, 1906, xxxiv, p. 169; Cannon and Nice: This journal, 1912, xxix, p. xxiv; Hoskins and McClure: Archives of internal medicine, 1912, x, p. 353; Elliott: Journal of physiology, 1912, xliv, p. 405.

[2] For a general discussion see Vincent: Internal secretion and the ductless glands, London, 1912.

[3] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 235.

[4] Langley: Journal of physiology, 1901–02, xxvii, p. 253.

[5] Badano: La Clinica Medica Italiana, 1898, p. 375.

[6] Boruttan: Pflüger's Archiv, 1899, lxxviii.

[7] Kahn: Archiv für physiologie, 1903, p. 530.

Methods

Cats and dogs were used in the experiments. The preparations were made in the following way. An animal was fastened back-downward to an animal holder and given urethane (2 gm. per kilo of body weight) by stomach. As soon as anesthetised, a cannula was inserted into a femoral artery, and a mercury manometer attached for recording the blood pressure. A second cannula was inserted into an external jugular vein deep down in the neck. Through this cannula the adrenalin solution was injected.

The abdominal cavity was opened by a median incision, and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall. From the S-shaped hook a thread was passed over a pulley to a writing lever which recorded the movements of the diaphragm on a revolving drum.

The movements of the chest wall were not recorded, as in a previous series of experiments on cats it had been found by one of us,[1] either to move synchronously with the diaphragm or not to move at all. Besides, comparative and not absolute depths of respiration were desired.

The adrenalin used was that of Parke Davis and Co., and was fresh. The strength of solution injected was chiefly a 1:100,000, made by diluting the 1:1000 stock solution with distilled water just before using. In some of the experiments, however, 1:50,000, 1:25,000 and even 1:1000 solutions were used. The injections, were made into an external jugular vein at a uniform rate (0.2 c.c. per second) by means of a small syringe having a graduated barrel.[2]

Results

The Relation of the Depth of Respiration to the Fall in Blood Pressure

When 0.3 c.c. or slightly less, of a 1:100,000 adrenalin solution was injected at a uniform rate (0.2 c.c. per second), into a cat,

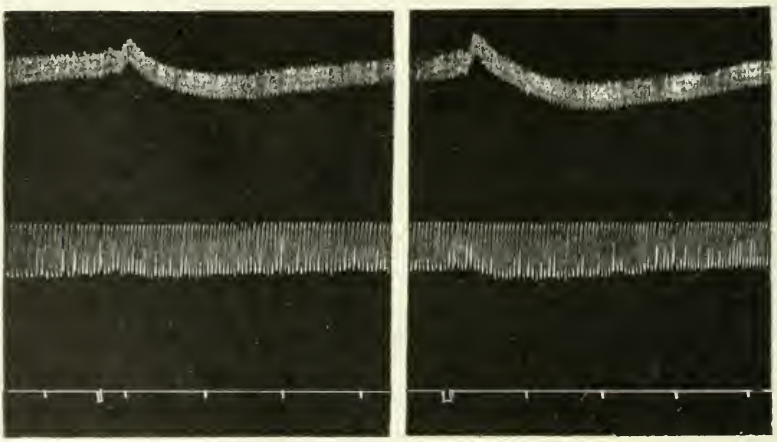
[1] Nice: This journal, 1914, xxxiii, p. 204.

[2] See Cannon and Lyman: This journal, 1913, xxxi, p. 376.

and 0.6 c.c., or slightly less, at the same rate into a dog, there invariably resulted a fall in blood pressure and an increase in the depth of respiration. In some cases the same results were obtained with larger doses. Before this increase there usually was a slight shallowness in the depth of respiration occurring almost immediately after the introduction of the solution. In some experiments, however, the increase in the depth was not preceded by shallowness.

The Relation of the Depth of Respiration to the Rise in Blood Pressure

In general 0.5 c.c., or more, of a 1:100,000 solution of adrenalin njected into the circulatory system of a cat at a uniform rate



A B

FIGURE 1. Cat A. In this and all following records the upper curve indicates the blood pressure; the middle curve the contractions of the diaphragm; and the lower line the time in half-minutes. At *a*, introduction of 0.3 cc. of 1:100,000 adrenalin solution; at *b*, introduction of 0.6 cc. of 1:100,000 adrenalin solution.

(0.2 c.c. per second) or 1.6 c.c., or more into a dog, at the same rate, produce a rise in blood pressure, and within limits, an increase in the depth of respiration. This increase in the depth of respiration, in a given animal, as well as the rise in blood pressure is proportional to the doses of adrenalin given, Figs. 1 and 2. The increases are nearly always preceded by a shallowness. The increase may be as much as 35 per cent (Fig. 3).

On the other hand, as Oliver and Schäfer[1] reported, if very large doses, as 0.3 c.c. of a 1:1000 solution of adrenalin or more, are

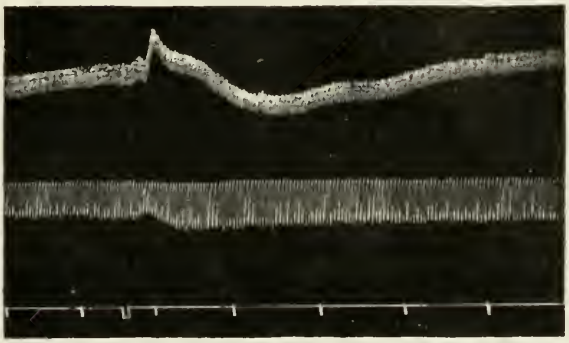


FIGURE 2. Cat A. Introduction of 1.2 cc. of 1:100,000 adrenalin solution.

injected into cats or dogs, the respiratory mechanism always responds by marked shallowness in breathing. For these large

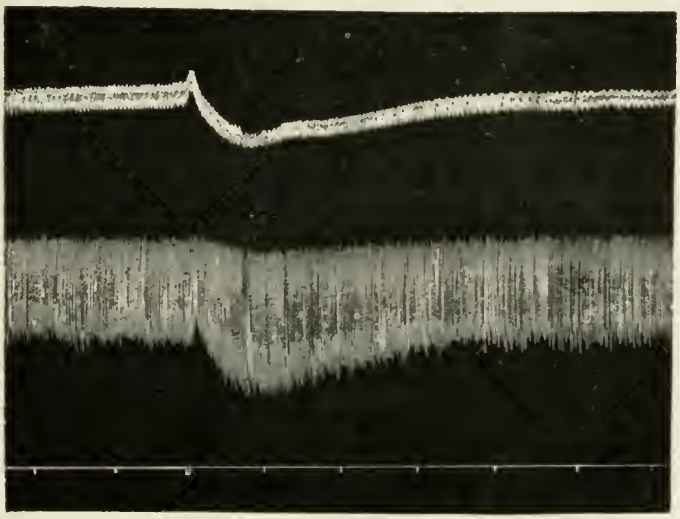


FIGURE. 3. Cat. Introduction of 0.35 cc. of 1:50,000 adrenalin solution.

doses, the decrease within limits, is in direct proportion to the dosage given and to the rise in blood pressure. In most cases the

[1] OLIVER and SCHÄFER: *loc. cit.*

shallowness was followed, as usual, by an increase in depth. In a few cases the respiration simply returned to normal.

Discussion of Results

The effect of adrenalin on the respiratory system seems to be due almost entirely to a central effect. Our results show that physiological doses have a stimulating effect on the center. Very large doses on the other hand have an inhibitory action on the respiratory center. These effects on respiration occur no matter whether the blood pressure is high or low. They also take place whether the vagi are intact or cut. By repeated doses the center becomes fatigued and the response is lessened. This is particularly true with large injections. When the breathing was irregular in some animals, the introduction of small doses of adrenalin made it regular.

Could the Increase in the Depth of Respiration be due in part to Direct Stimulation of the Diaphragm Muscle?

Considerable evidence has been brought forward to show that adrenalin does stimulate skeletal muscle to greater activity. Oliver and Schäfer injected adrenal extract into a frog and found that the excised muscle registered a curve of contraction about 33 per cent higher and 60 per cent longer than the corresponding muscle not subjected to the action of the extract.[1] Dessy and Grandis obtained a beneficial effect when adrenal extract was applied to fatigued muscle of a salamander.[2] Similar results were obtained by Panella.[3] Recently evidence was brought forth by Cannon and Nice,[4] and confirmed by Gruber,[5] to show that adrenalin indirectly improves the contraction of intact skeletal muscle in the cat by increasing the circulation through the muscle. Gruber's experiments show that the increase occurs only above a critical point in blood pressure, 90 to 100 mm. Hg. or above.

[1] Oliver and Schäfer: *loc. cit.*

[2] Dessy and Grandis: Archives italiennes de biologie, 1904, xli, p. 231.

[3] Panella: Archives italiennes de biologie, 1907, xlviii, p. 462.

[4] Cannon and Nice: This journal, 1913, xxxii, p. 44.

[5] Gruber: This journal, 1913, xxxii, p. 221.

We have been unable to find any increase in the contraction of isolated strips of diaphragm muscle, when the strip contracting in Ringer's solution in response to electrical stimuli was subjected to adrenalin.

Summary

1. The effect of adrenalin on respiration occurs synchronously with that on the circulatory system.

2. Doses of adrenalin which cause a fall in blood pressure elicit an increase in the depth of respiration. This increase may or may not be preceded by a shallowness.

3. Within limits, doses of adrenalin which produce a rise in blood pressure cause an increase in the depth of respiration. This increase, again, may or may not be preceded by a shallowness. The increase is proportional to the rise in blood pressure and to the amount of adrenalin given.

4. Excessive doses, as Oliver and Schäfer, and others have shown, produce a marked shallowness in breathing. Within limits, the shallowness is proportional to the effect on blood pressure and to the amount of adrenalin given.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

V. The Effects of Hemorrhage Before and After Exclusion of Abdominal Circulation, Adrenals, or Intestines

By H. GRAY and L. K. LUNT

[*From the Laboratory of Physiology in the Harvard Medical School.*]

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IN 1772 Hewson noted that in an animal bleeding to death the latest blood clotted more quickly than the earliest.[1] This was confirmed in 1842 by Nasse,[2] and in 1857 by Brücke.[3] Again in 1877 confirmation of the original observation was reported by Cohnheim in these striking words: "In a dog bled to death by the removal of blood from a vein in successive portions, the last portions often coagulate almost instantaneously." [4]

In 1901 Milian noted that in a hemorrhage following capillary puncture, i.e., finger or ear, the later drops had a progressively shorter clotting time till the latest, which as got by pressure had a quarter of the time of the earliest. From this he inferred that the shortening was due to a local influx of tissue juices.[5] Arloing promptly pointed out that in a hemorrhage following venous puncture there was the same progressive shortening, although there could be no local influx of tissue juices.[6]

Again in 1904 von Weismayr remarked the shortening after

[1] Hewson: An experimental inquiry into the properties of the blood, Experiment xxi, London, 1772, p. 69.

[2] Wagner: Handwörterbuch der Physiologie, Braunschweig, 1842, i, p. 75.

[3] Brücke: Archiv für pathologische Anatomie und Physiologie und für klinische Medizin, 1857, xii, p. 100.

[4] Cohnheim: Allgemeine Pathologie, 1877, p. 325; or Cohnheim's General Pathology, translated by McKee, London, 1889, p. 403.

[5] Milian: Comptes rendus Société de Biologie, 1901, liii, pp. 556, 576.

[6] Arloing: *ibid.*, p. 675.

hemorrhage.[1] And in 1909, Hartmann reported that the more bloody operations were generally but not invariably followed by decreased coagulation time. As to the causative factor he was unwilling to choose among diminished O_2, augmented CO_2, augmented fibrin ferment, and augmented flow of tissue thrombokinase proportionate to the size of the wound. Suddenness of the hemorrhage, however, he did define as essential to the decreased time, since he found like Schwab no decrease during the gradual exsanguination observed in myomatous women.[2] Two exceptional increases in such exsanguinated patients Hartmann viewed as due to hydration of the blood from the intestine, and in support of this view he cited [3] Terroine's repeated hemorrhages, followed by saline injections, which showed a primary decrease then a marked increase even to incoagulability.[4]

Later in 1909 von den Velden cited approvingly Nasse, Brücke, and their inference as to the independence of coagulation time and fibrin content.[5] Neither in them nor in Cohnheim nor in Hartmann could he find an adequate explanation, however; so he offered one reminiscent of them but in fact developed as an analogy to the explanation he had previously given for decreased clotting time after administration of halogen salts (bromides or chlorides). The decrease that he noticed after hemorrhage followed a loss of 19% of the circulating blood in rabbits, 8% in man. Incidentally this decrease was more marked in blood taken from veins than in that taken from capillaries. The explanation that he offered was Magnus' observation that small hemorrhages, i.e., up to 8 per cent, were followed by a thickening of the blood as measured by greater specific gravity,[6] and he gave confirming observations to show that this greater

[1] Schröder und Blumenfeld: Handbuch der Therapie der chronischer Lungenschwindsucht, Leipzig, 1904, p. 328.

[2] Schwab: Münchener medizinische Wochenschrift, 1906, liii, p. 2520; and 1907, liv, p. 176.

[3] Hartmann: Münchener medizinische Wochenschrift, 1909, lvi, p. 706.

[4] Terroine: Comptes rendus Société de Biologie, 1907, lxii, p. 143.

[5] R. von den Velden: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, pp. 37, 44.

[6] Magnus: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 104.

specific gravity occurred not only after but even during the hemorrhage. This thickening was followed by a secondary hydremia (thinning) with greater rapidity in proportion to the suddenness or size of the hemorrhage, as was shown by Zimmermann [1] and often since. The factor underlying this secondary hydremia was influx of tissue juices, as shown by Regeczy in 1885.[2] Hence the analogy between von den Velden's explanation of the decreased clotting time accompanying this hydremia, and his earlier explanation of the decrease accompanying osmotic hydremia (produced by a few c.c. of 10 per cent sodium chloride intravenously).[3] The common factor in these two analogous hydremias he accordingly assumed to be that coagulating substance (Thrombokinase, zymoplastische Substanz) long recognized as present in all tissue juices. He admitted that his explanation of decreased time as due to augmented thrombokinase was apparently inconsistent with Nasse's and Brücke's independent evidence of a decrease accompanied by diminished fibrin, but he considered them reconcilable.[4]

An attempt to throw light on the problem from another angle, namely localization of the factors in coagulation, led to the observations recorded below as to the effects of hemorrhage before and after exclusion of the abdominal circulation, of the adrenals, and of the intestines. The blood of 27 cats was studied by the method described previously [5] in this series. The size of the hemorrhages produced is stated as a percentage of the normal circulating blood, after that has been estimated as 8 per cent of the body weight. Secondary hemorrhages are stated similarly as percentages of the original blood, not as percentages of the blood left in circulation after the previous hemorrhage.

[1] Zimmermann: Archiv für physiologische Heilkunde, 1846, v, p. 349.

[2] Regeczy: Archiv für die gesammte Physiologie, 1885, xxxvii, p. 73.

[3] R. von den Velden: Deutsche medizinische Wochenschrift, 1909, xxxv, p. 197; Verhandlungen des Kongresses für innere Medizin, 1909, xxvi, p. 155.

[4] R. von den Velden: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, p. 42.

[5] Cannon and Mendenhall: This journal, 1914, xxxiii, p. 225.

Hemorrhage Decreases Clotting Time

The results arranged in Table I support Hartmann's idea that in order to hasten clotting hemorrhage must be moderately sudden or severe. This seems to mean about 13 per cent of the estimated body blood; *e.g.*, 7 per cent makes no change, but a second 7 per cent halves clotting time, and after it has returned to normal it is plainly decreased by a third 7 per cent (Dec. 4); and in other hemorrhages of 13 per cent and 12 per cent at the start the decrease is plain (Dec. 5 and Feb. 28). A second hemorrhage, in other words, either may produce a decrease after an initial hemorrhage has failed (Dec. 4), or may produce a further decrease (Dec. 5).

Further details for the experiments summarized in Table I, page 336, are given in Fig. 1 for the more typical results, and for the less typical in protocols (Dec. 4 and Feb. 28).

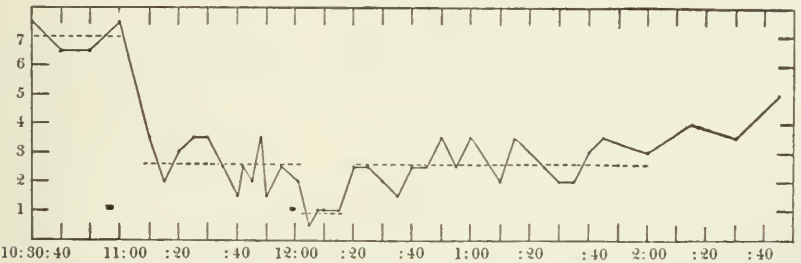

Figure 1. Record showing shortening of coagulation time after a hemorrhage (13 per cent of the blood) at 10:59, and after a second hemorrhage (10 per cent of the blood) at 11:59. The dotted lines in this and later figures indicate the averages for the time they cover.

Protocol (Dec. 4)

A cat weighing 3.3 k. was etherized at 9:20 A.M., at 10:15 decerebrated and given artificial respiration, at 10:55 the operation of preparing the femoral and carotid arteries was completed, an interval was left to allow a return to normal, after which the blood drawn at the moments stated showed the following clotting times:

11:26– 1.0 minutes	12:00– 2.5 minutes
11:29– 1.5 "	12:02– 1.0 (?) "
11:35– 1.0 "	12:10– 2.5 "
11:41– 0.5 "	12:15– 3.5 "
11:46– 3.0 "	12:20– 3.0 "
11:52– 2.5 "	12:25– 3.0 "

TABLE I

HEMORRHAGE DECREASES CLOTTING TIME (C.T.)

(In this and in later tables read from left to right along one line, then along the next line. This procedure will in many cases show on the next line a new clotting time without any new hemorrhage. This is so because a new line is used wherever the average clotting time for any group of tests varies markedly from the preceding average; e.g., On Dec. 4, the second hemorrhage of 7 per cent was followed by an interval of 2 minutes; in the next 7 minutes 3 samples were taken to test, giving an average of 1.5 minutes. Marked variation from this level is seen during the next 10 minutes by 3 samples whose average time was 3.5 minutes; hence a new line is used. Anomalous figures like 3.5 a are commented on in footnotes to each table.)

Date	C.T. before hemorrhage (Control C.T.)			Hemorrhage per cent	C.T. after hemorrhage			
	Duration of tests, minutes	No. of tests	Av. C.T. minutes		Interval minutes	Duration of tests, minutes	No. of tests	Av. C.T. minutes
Dec. 4 (Details in protocol)	15	4	1.0					
	24	5	2.3					
	10	3	3.2	7	1	10	3	3.2
				7	2	7	3	1.5
						10	3	3.5 a
				7	1	20	6	2.7
Dec. 5 (Details in Fig. 1)	30	4	7.0	13	11	51	13	2.6
				10	6	10	4	0.9
						75	16	2.6
						45	4	3.9
Dec. 16	15	4	3.0	12	1	45	8	4.5 b
				17	7	55	9	5.7
Feb. 28 (Details in protocol)	3	2	3.5					
	42	7	5.5	12	5	60	12	4.3
						60	3	5.5
						50	4	8.4a

(*a*) Secondary Rise: In many experiments the decreased coagulation time rises again not only toward normal as expected, but above. This secondary rise appears due in some cases to reaction in excess, so often remarked in biological processes when opposing factors are at work; and due in other cases to a curious progressive antemortem rise.

(*b*) Anomaly: Hemorrhage increased clotting time. This cat had been in the animal house for only three days and had been excited whenever approached. The coagulating factors roused by excitement may therefore have been exhausted (Cf. Cannon and Mendenhall: This journal, 1914, xxxiv, p. 249), so that hemorrhage was without effect.

12: 28 to 12: 29 Bled 20 c.c. from left carotid = hemorrhage of 7 per cent of body's blood.

12: 30– 3.5 minutes
12: 35– 3.0 "
12: 40– 3.0 "

12: 48 to 12: 49 Second hemorrhage 7 per cent.

12: 50– 2.0 minutes
12: 55– 1.0 "
12: 57– 1.5 "
1: 00– 3.5 minutes
1: 05– 3.5 "
1: 10– 3.5 "

1: 18 to 1: 19 Third hemorrhage 7 per cent.

1: 20– 3.5 minutes
1: 25– 2.0 "
1: 30– 2.5 "
1: 35– 3.0 "
1: 40– 2.5 " (Blood dark)
1: 45– 5.0 " (Blood dark; heart stopped)

PROTOCOL (Feb. 28)

A cat weighing 2.3 k. was etherized at 8: 00 A.M., at 8: 40 decapitated and given artificial respiration. At 10: 43 preparation of the femoral artery was completed, after which the clotting times were:

10: 45– 3.0 minutes
10: 48– 4.0 "
10: 54– 6.5 "
11: 05– 6.0 "
11: 12– 5.5 "
11: 20– 5.0 minutes
11: 25– 4.0 (?) "
11: 30– 6.0 "
11: 36– 5.5 "

11: 48 to 11: 50 Bled from femoral artery till muscular spasms = 23 c.c. = hemorrhage of 12 per cent.

11: 55– 4.5 minutes
12: 00– 4.0 "
12: 04– 4.5 "
12: 10– 4.5 "
12: 15– 4.0 "
12: 19– 3.5 "
12: 23– 4.5 minutes
12: 28– 5.5 "
12: 35– 4.5 "
12: 40– 4.0 "
12: 44– 4.0 "
12: 50– 4.0 "

12: 35 to 1: 35 Interval for lunch.

1: 37– 5.0 minutes
1: 42– 5.0 "
1: 50– 6.5 "
2: 00– 7.0 minutes
2: 15– 6.5 " (Blood dark)
2: 25– 10.0 " (Blood dark)
2: 40– 10.0 " (Blood dark; animal killed)

Where now can we localize the clotting factor or factors stimulated by hemorrhage?

Hemorrhage Fails to Decrease Clotting Time after Exclusion of the Abdominal Circulation

The earliest evidence that exclusion of the abdominal circulation increases coagulation time was given in 1886 by Stolnikow when he noted that blood circulating through heart and lungs did not coagulate in his apparatus as might be expected. He assigned as cause the fact that "the blood spent at most 20 seconds in the apparatus, after which it again was given over to the restorative influence of the vessel wall."[1] Pawlow in 1887 with similar technique noted further that the loss was gradual; i.e., blood tested when the experiment had been going on for only 15 minutes coagulated after a time, whereas blood "taken after a rather long experiment into a glass vessel showed no clot though kept till beginning decomposition." He inferred that the lungs produced some anti-clotting factor.[2]

Bohr in 1888 accepted Pawlow's experiments and inference, and supported them by shutting off the circulation at the diaphragm in two dogs and finding after about 15 minutes that carotid blood did not coagulate for twenty-four hours.[3]

In 1892 Lilienfeld thought Bohr's experiments so fundamental that he repeated them six times, and found that the time decreased twice and remained unchanged four times.[4] Prolongation of his six experiments might well have shown the more usual increase, to which the fall is only a prelude and that only sometimes (Table II *c*, March 9 and 11). His six findings are therefore quite reconcilable with ours, although we too must admit ignorance of the factor underlying that primary fall.

In 1895 Contejean objected that "the result of Bohr's experiments is hard to accord with the fact that blood from the subhepatic veins is normally almost incoagulable." But he gave no evidence. Furthermore, he repeated Bohr's experiments on dogs

[1] Stolnikow: Archiv für Physiologie, 1886, p. 10.
[2] Pawlow: Archiv für Physiologie, 1887, p. 459.
[3] Bohr: Centralblatt für Physiologie, 1888, ii, p. 263.
[4] Lilienfeld: Archiv für Physiologie, 1892, p. 152.

and cats without success . . . "the blood always remained coagulable" . . . "the blood coagulated without considerable delay." But in no instance did he give control evidence of clotting time prior to the exclusion of the abdominal circulation; and in only four instances did he give the time prior to routine experimental peptone infusions (which of course render all subsequent clotting times incomparable to Bohr's); these four were 6, 7, 14, and 17 minutes, which despite the lack of control do suggest some delay.[1] In general Contejean's effort was to "attribute to liver or intestine a preponderant part in producing the anticoagulating factor" which was active after peptone infusion, but he need not therefore have denied to liver and intestine a part in producing the normal coagulating factor. Several organs are already known to produce two different physiological factors.

In 1905 Nolf pointed out that although Doyon and Kareff were able several times to observe complete incoagulability they were obliged to sacrifice many animals. Hence he concluded that liver excision was not necessarily followed by complete incoagulability. In fact he added on the basis of numerous experiments of his own the conclusion that apart from special circumstances the incoagulability was not observed within the two hours that the dogs lived after liver excision. At the same time he admitted: "Increase of clotting time was usually noticed in the samples of blood taken after extirpation, but the clot looked normal and retracted strongly." This increase was accentuated if the operation was preceded by copious meat diet or if accompanied by portal stasis or if followed by hemorrhage. Like Pawlow he thought the increase gradual, and on this basis explained his failure to obtain the entire incoagulability previously observed by Doyon and Kareff.[2]

Although Nolf thought operative exclusion much more precise than toxic exclusion, he gave references to the accordant observations of many men who found either diminished fibrin or delayed coagulation after toxic liver injury, as by phosphorus. More extended observations of such effects were made in 1911 by Whipple and Hurwitz.[3]

[1] Contejean: Archives de physiologie, 1895, xxvii, pp. 248, 251.

[2] Nolf: Archives internationales de physiologie, 1905–06, iii, pp. 1, 7, 8.

[3] Whipple and Hurwitz: Journal of experimental medicine, 1911, xiii, p. 136.

The results arranged in Table II support (1) the inference of several of the above investigators that exclusion of the abdominal circulation excludes the clotting factor or factors and therefore

TABLE II

(1) Exclusion of the Abdominal Circulation (E) Increases Clotting Time (C.T.)
(2) Subsequent Hemorrhage No Longer Decreases Clotting Time

Date	C.T. before exclusion			Exclusion by tying vessels above diaphragm (E)	C.T. after exclusion				Hemorrhage per cent	C.T. after exclusion + hemorrhage			
	Duration of tests, min.	No. of tests	Av. C.T. min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T. min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T. min.
Dec. 13 (Details in Fig. 2)	15	4	4.2	E	2	55	10	5.1	5	2	56	10	5.3
									5	7	20	3	8.7
										12	24	5	5.7
Mar. 9	23	4	6.5		8	11	1	7.5					
						8	2	6.5					
				E		11	2	5.0c					
						57	4	8.2					
Mar. 11 (Details in protocol)	59	7	6.6	E	2	75	8	7.7	11	1	14	3	7.8

(c) This one instance of secondary brief decrease remains to be explained. Possibly as Pawlow suggested "if one compares . . . the coagulation effect of thymus . . . extract discovered by Wooldridge, one must suspect that the condition usually present in the blood should be regarded as a resultant of several mutually opposed factors arising from different organs." One of these factors is without doubt the blood platelets whose "importance in coagulation has been recognized since the work of Bizzozero and Hayem about 1880" (Lee and Vincent: Archives of internal medicine, 1914, xiii, p. 404), and whose origin has been localized by Wright in the giant cells of the bone marrow (Wright: Journal of morphology, 1910, xxi, p. 270).

increases clotting time; and also support (2) Nolf in that hemorrhage subsequent to exclusion no longer decreases the time as in animals with normal circulation, and may even accentuate the

increase previously produced by exclusion. The method here used of excluding abdominal circulation was compression (clamp or ligature) of aorta and cava just above the diaphragm, making a more completely "anterior animal" than that of Stolnikow, Pawlow, Bohr, Lilienfeld, and Contejean; though less completely anterior than that of Whipple.

Further details of the experiments summarized in Table II are given in Fig. 2, for the more typical results, and for the less typical in a protocol (Mar. 11).

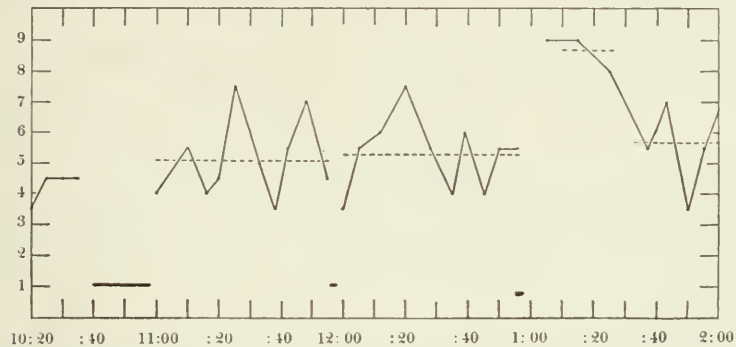

FIGURE 2. Record showing absence of rapid clotting after hemorrhage, when the circulation is confined anterior to the diaphragm. From 10:40 to 10:58 the operation of tying the aorta and inferior cava above diaphragm was performed. At 11:58 5% of the blood was drawn, and at 12:58 5% again, each time with resulting respiratory distress.

PROTOCOL (Mar. 11)

A cat weighing 2.5 k. was etherized at 8:55 A.M., at 9:15 decerebrated, at 9:25 artificial respiration. Hemostats were clamped on aorta and cava just above diaphragm, removed, applied again, removed again. While they were on the clotting time was lengthened, and shortened again after their removal. The actual figures varied much, apparently because of the shock caused by the procedure. Hence the figures are not reproduced, except for the latter part of the experiment, when the aorta and cava were not clamped but ligated (at 2:31 P.M.).

2:33– 7.5 minutes	3:23– 7.5 minutes
2:42– 6.0 "	3:31– 8.0 "
2:48– 8.5 "	3:40– 7.5 "
3:12– 8.0 "	3:48– 8.5 "

4: 11 to 4: 12 Drew 11 c.c. from carotid = 11 per cent of blood circulating in this anterior animal.

4: 13– 8.0 minutes
4: 21– 6.0 "
4: 27– 9.5 minutes
4: 37 (No blood obtainable; dead)

Hemorrhage Decreases Clotting Time after Removal of the Adrenal Glands

Having localized the clotting factor or factors in the abdomen we shall now attempt to make the localization more definite. That the adrenal glands may play a part in the clotting which follows hemorrhage was suggested by the observations that augmented adrenalin percentage in the blood hastens coagulation,[1] and that just such hyperadrenalinemia follows strong sensory stimulation,[2] which is the usual accompaniment of trauma (accidental, military, surgical) and its attendant hemorrhage.

In 1911 Trendelenburg stated that when the blood pressure was diminished by vigorous hemorrhage (7–17 per cent), the absolute amount of adrenalin secreted was maintained, i.e., there was an absolute decrease but a percentage increase of adrenalin in the blood.[3] On these facts he denied "regulation of diminished blood pressure by adrenal hypersecretion." Still his evidence of adrenal hypersecretion after hemorrhage agrees with our experience of faster clotting after hemorrhage. Incidentally it may here be noted that his denomination of 7–17 per cent as a vigorous hemorrhage accords remarkably with our experience that 7–14 per cent was the maximum possible, varying according to the animal, without producing extreme air-hunger and muscular spasms.

The question now arises, are the adrenals essential to the more rapid clotting after hemorrhage? The figures presented in Table III show that even after removal of the adrenals, hemorrhage decreases clotting time. In two instances (Feb. 18 and Mar. 2) it was noted that removal of the adrenals was followed by faster clotting — a result probably due to expression of adrenalin,[4]

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

[2] Cannon and Mendenhall: This journal, 1914, xxxiv, p. 251.

[3] Trendelenburg: Zeitschrift für Biologie, 1911–12, lvii, p. 98.

[4] Stewart: Journal of experimental medicine, 1912, xv, p. 547; and Hoskins and McPeek: Journal of the American Medical Association, 1913, lx, p. 1778.

which cannot be wholly obviated by even the most careful avoidance of massage (by gentle handling; and by tying off the lumbo-adrenal vein at its entrance to the gland and at its exit, as well as the gland pedicle with its vessels). In neither of these cases did the clotting process after the removal become slower than before. Thus the observations here recorded show that the adrenals are not the sole nor even the major factor, because after their removal coagulation time is not lengthened.

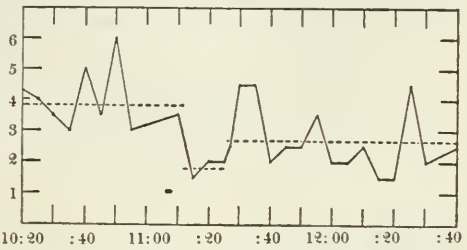

FIGURE 3. Records showing shortening of coagulation time after hemorrhage (13 per cent of the blood) at 11:08, though the adrenal glands had been previously (8:55–9:05) removed.

Further details for the experiments summarized in Table III, page 344, are given in Fig. 3 for the more typical results, and for the less typical in protocols (Jan. 7 and Mar. 2).

PROTOCOL (Jan. 7)

A cat weighing 2.4 k. was etherized without a struggle at 2:30 P.M., at 3:05 the adrenals were tied off (afferent and efferent lumbo-adrenal vein and also the pedicle), at 3:20 animal decerebrated.

3:58– 7.0 minutes
4:05– 4.0 "
4:10– 3.0 "
4:14– 4.0 minutes
4:19– 4.5 "

4:26 Pithed cord through orbit. Artificial respiration.

4:28– 3.5 minutes
4:33– 6.0 "
4:40– 4.0 minutes
4:51– 4.5 "

5:03 to 5:05 Bled till air-hunger = 35 c.c. = 18 per cent of estimated blood volume.

5:06– 3.0 minutes
5:15– 4.0 "
5:20– 2.5 "
5:25– 2.5 "
5:29– 6.0 "
5:35– 5.5 "
5:45– 9.0 minutes
6:00– 7.0 "
6:08– 7.0 "
6:15– 6.0 "
6:21 (No blood obtainable; dead)

TABLE III

HEMORRHAGE AFTER REMOVAL OF THE ADRENAL GLANDS (R) DECREASES CLOTTING TIME (C.T.)

Date	Removal of adrenals (R)	C.T. before hemorrhage				Hemorrhage per cent	C.T. after hemorrhage			
		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.
Dec. 6 (Details in Fig. 3)	R	65	55	10	3.8	13	7	10	3	1.8
								73	15	2.7a
Dec. 17	R	30	35	5	5.3					
			15	3	4.0	8	7	48	7	5.5d
						6	5	35	7	3.7
Dec. 18	R	25	85	14	3.0	20	1	10	2	2.0
Jan. 7 (Details in protocol)	R	53	7	1	7.0					
			14	4	3.9					
			23	4	4.5	18	1	19	4	3.0
								46	6	6.7a
Feb. 18	R	5	5	2	4.0					
			240	24	5.4	5	1	25	4	8.0e
Mar. 2 (Details in protocol)	R	5	27	5	5.7					
			64	7	6.7	14	5	9	1	8.5f
								20	4	4.9
								65	9	6.6a
						9	5	24	5	7.0e

(*a*) cf. Table I.

(*d*) This 5.5 though higher than 4.0 is so little higher than 5.3 or than their average 4.8 that it very likely lies within the limits of error and means only that the small hemorrhage had no more effect than on Dec. 4, Table I. Note that, as on that date, a second hemorrhage produces the expected decrease.

(*e*) This increase may mean either that the hemorrhage had no more effect than on Dec. 4, Table I, so that the preceding secondary rise (e.g., on Feb. 18 secondary to the primary fall 4.0. The control average clotting time before removal of the adrenals was 6.2 minutes) simply continued uninterrupted; or it may mean an anomaly yet to be explained.

(*f*) This increase may mean either of the two possibilities under *e* (the average clotting time before removal of the adrenals was 6.9 minutes); or thirdly, being a single test, may mean an artefact due to leakage from the water bath through the rubber connection which was somewhat old and was in this case found to have become suddenly loose, probably due to too long rinsing in the beaker of ether.

PROTOCOL (Mar. 2)

A cat weighing 2.1 k. was etherized at 9: 55 A.M., without a struggle, at 10: 20 decerebrated, at 10: 35 preparation of femoral artery completed.

10: 40– 5.0 minutes
10: 47– 5.0 "
11: 00– 5.5 "
11: 10– 7.0 "
11: 20– 6.5 minutes
11: 30– 7.0 "
11: 40– 7.0 "

11: 45 to 12: 10 Removal of adrenals.

12: 15– 7.0 minutes
12: 23– 6.5 "
12: 30– 5.5 "
12: 36– 5.0 "
12: 42– 4.5 "
12: 55– 6.0 "
1: 02– 7.0 minutes
1: 26– 7.5 "
1: 34– 7.0 "
1: 42– 6.5 "
1: 50– 7.0 "
1: 59– 6.0 "

2: 09 to 2: 11 Bled from femoral artery till muscular twitching (not till spasms) = 24 c.c. = hemorrhage 14 per cent of circulating blood.

2: 16– 8.5 minutes
2: 25– 4.5 "
2: 30– 5.5 "
2: 38– 4.0 "
2: 45– 5.5 "
2: 52– 7.5 "
3: 01– 6.5 "
3: 08– 8.0 minutes
3: 22– 7.5 "
3: 30– 5.0 "
3: 40– 6.0 "
3: 47– 5.5 "
3: 57– 6.0 "
4: 09– 7.5 "

4: 21 to 4: 22 Bled 15 c.c., no spasms resulting nor air-hunger = hemorrhage 9 per cent.

4: 25– 7.0 minutes
4: 32– 7.0 "
4: 40– 6.0 "
4: 49– 7.0 minutes
4: 57– 8.0 "

5: 15 Pithed cervical cord through orbit; artificial respiration.

5: 20– 11.0 minutes
5: 32– 6.5 "
5: 40– 5.5 "
5: 46– 4.5 minutes (Blood dark; pressure low)
5: 51– 7.5 minutes (Blood dark; pressure low)
6: 00 (No pressure; dead)

Hemorrahge Decreases Clotting Time after Exclusion of the Intestines

In 1899 Mathews[1] published an experiment in which tying of the superior and inferior mesenteric arteries and the mesenteric vein in a cat was followed by marked increase of the coagulation time. Although Brücke stated that the shortening after hemorrhage was not paralleled by abundance of fibrin, and although similarly Mathews showed convincingly that the lengthening after exclusion of the intestine was not paralleled by absence of fibrinogen, still it is interesting to observe that the long-standing belief in the parallelism between the rate of clotting and the quantity of fibrinogen is supported by the agreement of the experiments here reported with a note by Goodpasture earlier this year that ligature of the intestine or cutting off half the blood to the liver caused a marked delay in fibrinogen reproduction after complete defibrination.[2]

Goodpasture quoted Bohr's and Mathews' evidence for incoagulability after exclusion of intestine, and in opposition gave only the general statement that in his experiments "specimens taken at frequent intervals during the perfusion have nearly always clotted within the normal time, one to seven minutes." It seems as if with less liberality as to the range of normal time he might well have found a significant variation in coagulation time, whether increase or decrease.

The results presented in Table IV support (1) both Bohr and Mathews by showing that exclusion of the small and half the large intestine increases clotting time. This increase is preceded by no such primary decrease as occurred immediately after adrenalectomy, and this is as might be anticipated from the fact that the circulation through the intestines can be easily cut off without massage of the adrenals or other viscera. The intestine, therefore, like the adrenal gland is a factor in clotting. But, again like the adrenal, it is not the sole nor even the major factor, because, as the Table further shows, hemorrhage after exclusion of the intestines can still cause a decrease in clotting time.

[1] Mathews: This journal, 1899, iii, p. 79.

[2] Goodpasture: This journal, 1914, xxxiii, p. 85.

TABLE IV

(1) Excision of the Small and Half the Large Intestines (E) Usually Increases Clotting Time (C.T.)

(2) Subsequent Hemorrhage Decreases Clotting Time

Date	C.T. before excision			Excision	C.T. after excision				Hemorrhage per cent	C.T. after excision + hemorrhage			
	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.
Dec. 11 (Cf. protocol)				E	15	11	3	4.0	9	2	34	9	3.7
											10	3	4.5
									6	3	20	6	2.7
Jan. 8 (Cf. protocol)				E	25	10	3	4.8	13	2	17	3	6.3g
											48	7	3.9
									8	2	3	1	2.5
											9	3	5.2a
Feb. 20	58	6	10.6	E	10	11	2	12.2	?	3	20	2	7.5
Feb. 21 (Cf. Fig. 4)	35	5	7.0	E	8	37	5	8.8					
					65	10	2	9.7	10	5	36	6	5.4
Mar. 3	54	7	6.1	E	5	15	4	4.5h					
						5	2	5.7	15	5	19	4	4.9
												1	7.5a

(*a*) cf. Table I.

(*g*) Anomaly: In one case, hemorrhage after excision of the intestine caused, before the usual decrease, a marked increase of coagulation time. This primary increase may indicate that excision of the intestine removes the major coagulating factor, and that some time must elapse before the substitute factor gets to work in making the usual decrease.

(*h*) Anomaly: In one case, excision of the intestine, after tying through and injuring the pancreas instead of removing it intact, caused a decrease. Responsibility lies therefore to all appearances in some way with the pancreas, although such a conclusion appears contrary to the experiment of Mathews cited above on p. 346.

Further details for the experiments summarized in Table IV, page 347, are given in Fig. 4 for the more typical results, and for the less typical in protocols (Dec. 11 and Jan. 8).

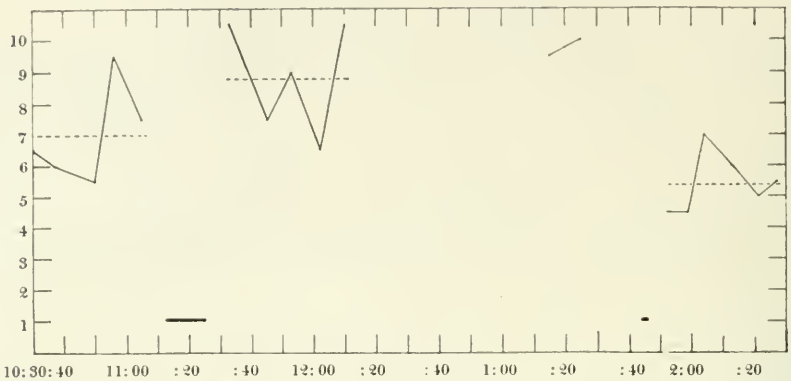

FIGURE 4. Record showing lengthening of coagulation time after removal of the small and half of the large intestine (11:12–11:25), with shortening of the coagulation time after subsequent hemorrhage (10 per cent of the blood) at 12:46.

PROTOCOL (Dec. 11)

A cat weighing 3.3 k. was etherized at 8: 25 A.M.; at 8: 35 vessels tied off in mesentery near intestine; at 8: 45 the small and half of large intestine excised; at 8: 50 preparation of the femoral artery completed; and at 8: 55 the animal was decerebrated.

9: 00– 4.5 minutes
9: 05– 6.0 "
9: 11– 5.5 "
9: 17– 5.0 "
9: 23– 4.0 minutes
9: 28– 4.0 "
9: 34– 4.0 "

9: 39 to 9: 40 Bled 23 c.c. = 9 per cent of total estimated blood.
9: 41– 4.5 minutes (Blood dark)
9: 46– 3.0 " (" ")
9: 50– 2.5 " (" normal)
9: 53– 5.0 "
9: 58– 4.0 "
10: 03– 4.5 minutes
10: 08– 3.5 "
10: 12– 3.0 "
10: 15– 4.0 "

10: 19 to 10: 29 Tied off gastro-hepatic omentum except hepatic artery.
10: 20 Artificial respiration.
10: 30– 4.5 minutes
10: 35– 4.5 "
10: 40– 4.5 "

10: 52 to 10: 53 Bled 15 c.c. = 6 per cent.

10: 55– 3.0 minutes
10: 58– 2.0 "
11: 00– 2.0 "
11: 05– 3.0 minutes
11: 08– 4.5 "
11: 15– 2.0 " (Blood dark; heart stopped)

PROTOCOL (JAN. 8)

A cat weighing 3.7 k. was etherized with a marked period of excitement at 2: 20 P.M., at 2: 50 decerebrated and given artificial respiration; at 2: 55 vessels tied off in mesentery near intestine, the small and half the large intestine excised; at 3: 05 abdomen closed with clamps.

3: 20– 4.5 minutes
3: 25– 5.0 "
3: 30– 5.0 "

3: 41 to 3: 42 Bled till air-hunger = 38 c.c. = 13 per cent.

3: 43– 7.5 minutes
3: 50– 5.5 "
4: 00– 6.0 "
4: 07– 4.0 "
4: 15– 3.5 "
4: 25– 3.0 minutes
4: 35– 4.0 "
4: 40– 4.0 "
4: 45– 5.5 "
4: 55– 3.5 "

5: 01 to 5: 02 Bled till air-hunger = 24 c.c. = 8 per cent.

5: 03– 2.5 minutes
5: 06– 4.0 "
5: 10– 4.5 "
5: 15– 6.5 minutes
5: 23 (No blood obtainable; dead)

DISCUSSION

The results presented in the foregoing pages confirm earlier observations by showing that clotting time is shortened by hemorrhage when that is sudden and of sufficient amount (at least 13 per cent of the blood volume). That this effect is due to some change in the abdominal viscera is indicated by a failure of the blood to clot faster when hemorrhage occurs after the circulation is confined anterior to the diaphragm. Previous experimentation has pointed to the adrenal glands, the intestines, and the liver, as organs in the abdomen which are concerned with clotting.

As proved by an earlier paper in this series stimulation of the adrenal glands results in more rapid clotting.[1] From Trendelenburg's evidence, previously cited, that the percentage of adrenalin in the blood is increased after hemorrhage it is probable that the faster clotting which follows bleeding is due to effects on the adrenal glands, in part. On the other hand the shortening of coagulation time by hemorrhage after the adrenal glands have been removed proves that they are not an essential factor in the phenomenon.

Several earlier investigators have studied the intestine-liver complex. In 1888 Bohr noticed that exclusion of the intestine and liver by tying splanchnic arteries produced after four hours no untoward symptoms but a blood which after withdrawal showed no clot until more than two hours had passed and even then it was abnormal, small and soft.[2] In 1899 Mathews published three experiments in which exclusion of intestine and liver by tying most of the splanchnic vessels was followed by marked increase from about 2 minutes to 30; and one experiment in which excision of the spleen and pancreas was followed by no increase. The fibrinogen content, however, did not change, and the clot became complete only after hours (as Hammarsten and Schmidt had shown to occur in solutions of fibrinogen poor in ferment), wherefore he ascribed the increase "probably to a diminution of fibrin ferment."

The experiments in which we removed the small intestine and half the large, support the evidence adduced by several previous workers that the intestines are probably an important agency in providing a factor or factors favorable to blood clotting. But they too are not essential, for after they have been thus eliminated, hemorrhage still shortens the coagulation time.

The liver is left for consideration, Its importance for coagulation is well established. In 1904 Doyon and Kareff excised the liver in a dog whose blood coagulated in 3 minutes and joined the portal to a subhepatic vein, *i.e.*, cava. After this, one specimen coagulated in 8 minutes, one in 20, and two not at all.[3] They

[1] Cannon and Mendenhall: This journal, 1914, xxxiv, p. 243.

[2] Bohr: *loc. cit.*

[3] Doyon and Kareff: Comptes rendus Société de Biologie, 1904, i, p. 612.

confirmed the observation several times.[1] In 1912 Meek found that after an Eck fistula fibrinogen regenerated more slowly than normally,[2] and similarly Goodpasture noted this year that cutting off half the blood to the liver caused a slight but noticeable reduction in the rate of fibrinogen regeneration after complete defibrination.[3] In two experiments (not here detailed) we found, like previous observers, that exclusion of the liver prolongs coagulation time.

On the basis of evidence given above it is probable that of the organs in the abdomen affecting the clotting time of blood those most directly concerned with faster coagulation after hemorrhage are the intestines and liver. Certainly the results we have obtained show that the liver is capable of producing this hastening in the absence of the intestines. Our experiments have not permitted us, however, to testify whether the liver is or is not more effective than the intestines in the shortening of coagulation time which follows rapid withdrawal of blood from the body.

Summary

I. Hemorrhage decreases clotting time, especially if moderately severe — 13 per cent of the circulating blood.

II. The most important coagulating factors may be localized in the abdomen, because: (1) exclusion of the abdominal circulation increases clotting time, and (2) hemorrhage after exclusion no longer stimulates a decrease of clotting time.

III. The adrenal glands probably favor by their secretion rapid clotting after hemorrhage, but after adrenalectomy hemorrhage still decreases the clotting time.

IV. Exclusion of the intestines usually increases clotting time, but after this exclusion hemorrhage can still decrease the clotting time.

V. The important rôle which the liver has been proved to play in coagulation indicates that it provides, perhaps in cooperation with the intestines and the adrenal glands, the necessary elements to hasten clotting after extensive bleeding.

It is a pleasure to acknowledge our indebtedness to Dr. W. B. Cannon for his advice and aid.

[1] Cited by Nolf: *loc. cit.* [2] Meek: This journal, 1912, xxx, p. 161.
[3] Goodpasture: *loc. cit.*, p. 84.

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OBSERVATIONS ON THE TOXIN OF *RHIZOPUS NIGRICANS*

By ROSS AIKEN GORTNER and A. F. BLAKESLEE

[*From the Biochemical Laboratory of the Station for Experimental Evolution, The Carnegie Institution of Washington*]

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Introduction

In a preliminary note [1] we have shown that the "presssaft" from *Rhizopus nigricans* contains a powerful toxin. At that time we had not attempted to prepare the toxin in a more concentrated form and had only ascertained that an aqueous extract from 0.045 gram of the dried mould filaments or mycelium was sufficient to kill a 1.35 kilo rabbit in two minutes when the toxin was injected intravenously.

In this paper we desire to present some further observations on the chemical nature of this toxin, methods of obtaining a more concentrated preparation and the results of physiological tests. Although we believe that we have not, as yet, isolated the toxic principle in a pure state, we have obtained preparations having a lethal toxicity of approximately 1:275,000 parts of body weight when injected intravenously into rabbits. Inasmuch as one of us (G.) must of necessity abandon further work on this subject we are presenting the results more recently obtained.

Experimental

1. *Physiological Tests using Extracts of the Mycelium or Aerial Filaments*

(*a*) **Intravenous Injections.**—The presence of a toxin in the "presssaft" of *Rhizopus* was noticed during a series of immunization experiments having as their aim a chemical analysis of the cause of sex in the Mucors.[2] The aerial filaments of the mould were ground with sand and the juice expressed by means of a small beef juice press and centrifuged at high speed to remove suspended solids. When 2 c.c. of such an extract is injected intravenously into the ear of a rabbit death is almost instantaneous, the animal often expiring before the injection is complete (about 5 seconds) or before the needle can be withdrawn from the vein. In such a case there are no typical symptoms accompanying death, there is rarely any struggle and only a sudden sinking of the head to one side and a convulsive twitching of the body.

When, however, only about *one tenth* of this dose is given (diluted to 2 or 3 c.c. with physiological salt solution) the symptoms are invariably the same. A few seconds after the needle is withdrawn the head sinks to one side and a violent convulsive spasm follows. This may be followed by a "typical death" (see below) or by a short rest period followed by another convulsive spasm, depending both on the size of the dosage employed and also on the degree of resistance possessed by the animal.[1] The convulsive spasms rarely last longer than 30 seconds while the rest period between may be as long as 30 minutes. During the rest periods the animal usually lies fully stretched out, the only indication of life being its regular breathing. At the close of the final spasm there is usually a short rest period of 5 to 10 seconds followed by a spasmodic cough-like movement of the diaphragm with widely opened mouth as if gasping for breath. There are usually 8 to 10 of these cough-like movements of the diaphragm, each shorter and more rapid than the one preceding. During this period the head

[1] We have observed considerable individuality in these animals; in some instances one animal would be killed almost instantly while with the same dosage another animal of like weight would live for 15–20 minutes.

is thrown backward and the chest and abdomen are distended and turgid, so much so that only by strong pressure can the wall of the abdomen be flexed. This reaction usually lasts 8 to 12 seconds and is followed by a relaxation of the rigid abdomen, protruding eyes and death. This description of a death is meant wherever a "typical death" may be referred to later. All of these symptoms may be shown within a period of 15 seconds from the time the injection is made or death may be deferred for several hours. In the latter case the behavior of the animal is usually "normal" until within a few minutes before death. Such a condition accompanies a dosage *just sufficient* to cause death.

When the dosage is *just sub-lethal* there is usually a short convulsive spasm followed by a long period (24 to 48 hours) of extreme lethargy, and complete recovery. In nearly all instances tested a fall of nearly 4° in the body temperature of the rabbit occurred during the first 12 hours after receiving the injection, and as recovery progressed the temperature gradually rose. Since the rabbit has no constant temperature it is impossible to determine the significance of this factor with exactness, but it seems probable that a fall from 102.4° to 98.5° is significant, and such a fall has been observed in several instances.

No tolerance was observed when an animal which had received a just sub-lethal dose was again given a similar injection after complete recovery. On the contrary the symptoms were slightly more violent than in the first injection.

When a *slightly* smaller dose than just sub-lethal is injected *there is usually no noticeable reaction*, the animal remaining to all appearances normal. If, however, after 30 to 60 minutes, a slight additional quantity is injected *just sufficient to raise the original dosage within the lethal limits*, there is a sudden violent reaction and death within a few seconds or minutes.

(*b*) **Subcutaneous Injections.**—Only four experiments were made where the "presssaft" was injected subcutaneously, but since each of these injections gave similar results it was thought unnecessary to prolong the investigation further. In each instance the injection was made under the skin of the back under aseptic precautions. The "presssaft" was sterilized either by rapidly heating the solution to *just boiling* and then cooling quickly or else by the addi-

tion of ether or chloroform in a sterile tube, plugging with sterile cotton and evaporating the antiseptic under diminished pressure.

A typical instance of the results is as follows: Rabbit No. 60, weight 1330 grams, received subcutaneously 6.7 c.c. of the "presssaft" at 11 A.M. May 21. This dosage was the equivalent of about 20 intravenous lethal doses. No results were observed during the first 24 hours. After several days it was noticed that the skin seemed loose and the flesh "flabby" but no other symptoms were observed. Eight days after the injection a small purulent ulcer was observed on the belly of the rabbit and the weight had fallen to 1050 grams. Although the ulcer was well washed with "peroxide" and bound up with iodoform gauze it increased in size and inasmuch as it showed no sign of healing the animal was chloroformed 12 days after making the injection. The weight at death was 850 grams.

This result would seem at first glance to be due to bacterial infection at the time the injection was made, but we do not believe this to be the case, for in numerous instances we have injected subcutaneously the "presssaft" of other moulds (as well as physiological salt solution) with far less precaution against infection and have never had such injection followed by either lesions or by a considerable loss in weight.

On examination we found that there was an open channel from the site of the sore to the point of injection and subsequent investigation in other cases showed that the "presssaft" is not absorbed (or else that serum exudes) so that the lump formed by the mass of liquid under the skin gradually works down the side of the animal until it reaches the belly. This movement of the liquid under the skin is probably brought about by the action of gravity, possibly combined with a corrosive action of the toxin. After reaching the belly the mass of liquid can be distinctly felt under the skin for 2 to 3 days, at the end of which time the skin is eaten through from within and the ulcer begins.

(*c*) **Administration of the Mould per Os.**—Rabbits, guinea pigs, and chickens were fed the moist mycelium or the dried and ground mycelium and aerial filaments of *Rhizopus*. In only one instance was there any noticeable effect. A guinea pig weighing 705 grams

was fed one gram daily of the dried mould mycelium in gelatin capsules on five consecutive days. There was a marked loss of weight each day and at the end of the sixth day the weight had fallen to 505 grams, showing a loss of nearly 30 per cent of the original body weight. Unfortunately at about this time one of the contagious diseases to which guinea pigs are subject developed in the animal house and in the course of the next 10 days all of the pigs had died. We are unable, therefore, to say with certainty that this loss in weight was due to the feeding of the mould although we believe that such may be the case, since the animal was apparently perfectly normal in its actions during the feeding experiment and also since it was one of the last of the animals to die.

Rabbits were fed upon bread upon which a heavy culture of the mould was growing and also upon corn meal upon which *Rhizopus* had been grown for a week or more. No ill effects of such feedings were observed.

2. *Autopsy after Death following an Intravenous Injection*

Dr. O. T. Avery of the Hoagland Laboratories, Brooklyn, was kind enough to witness the symptoms caused by an intravenous injection of the toxin into both rabbits and guinea pigs and also to assist in an autopsy. The only abnormal conditions apparent were a pronounced heart block, the auricles beating about 3:1, and a partial inflation of the lungs. Avery has investigated the effects of the toxin prepared from the tubercle bacilli by the method of Vaughan [3] as well as the toxic properties of the split products of edestin [4] and he pronounced the death symptoms and the autopsy following *Rhizopus* injection as being "typical" of toxins of this type, and practically indistinguishable from an anaphylactic intoxication. Unless we assume that all animals are born "sensitive" to *Rhizopus* it will be almost impossible to call the death anaphylaxis. On the other hand the preparation of the toxin precludes any splitting of the protein molecules such as is brought about by Vaughan's hydrolysis with alkali. It seems possible however that *Rhizopus* so utilizes the protein of the culture medium that a split product having toxic properties similar

to the toxins prepared by Vaughan's method is present in the "presssaft."

3. *Attempts to Isolate the Chemical Substance Responsible for the Toxic Action*

About 5 c.c. of the "presssaft" was evaporated to dryness *in vacuo* over sulphuric acid and extracted first with warm ether and then with warm alcohol. The ether and alcohol were evaporated in a dessicator and the residue taken up with physiological salt solution and injected intravenously into a rabbit. No ill effects were observed. It was later found that the toxic principle could be completely precipitated from its aqueous solution by the addition of four volumes of 95 per cent alcohol. Following this observation a concentration of the toxin was obtained by two methods.[1]

Method. (*a*)—The mycelium of *Rhizopus*, grown on solid agar medium under conditions of extreme moisture with a consequent nearly complete suppression of sporangial formation, was stripped

[1] Since preparing this paper for publication our attention has been called to the work of Paladino-Blandini.[5] Paladino-Blandini tested *Rhizopus*, together with other genera, for the presence of a toxic phenol by extracting the dried mycelium with 90 per cent alcohol evaporating the extract to dryness, and injecting a suspension of the dried residue. In *Rhizopus* three injections of 0.05 grams each, spaced at an interval of half an hour, were necessary to kill a 3 ko. rabbit. The death symptoms of the rabbit were very similar to those which we have observed with our toxin. We have, however, injected 0.108 gram of the alcohol soluble substances (extracted from the aerial mycelium with *absolute* alcohol in a Wiley extraction apparatus) into a 1200 gram rabbit without ill effects. It seems possible that extraction with 90 per cent alcohol may dissolve a part of our toxin, or, which seems still more probable, that some of the toxin formed a colloidal solution with the 90 per cent alcohol. This will occur whenever alcohol is added to a solution of the toxin in the absence of electrolytes. Another possible cause of death is embolism due to the very considerable quantity of insoluble lipins injected into the vein.

It is certain that Paladino-Blandini had only a small part of the toxin present in his *Rhizopus* mycelium, for we have found that the alcohol soluble material does not exceed 30 per cent of the dry weight of the fungus, therefore to obtain 0.15 gram of the alcohol soluble portion would require 0.50 gram of the dry mycelium, the aqueous extract of which would contain sufficient toxin to kill *five* 3 kilo rabbits *within two minutes* after the injection.

off, cleaned from agar, dried over sulphuric acid *in vacuo* and powdered.[1]

The powdered mould in 25 gram lots was poured into 200 c.c. of boiling water and stirred for about 30 seconds, then filtered on a Buchner funnel with suction. This process was repeated on the residue twice more with fresh hot water, the filtrates were united and concentrated at 50° to 55° under a pressure of about 30 mm. to about 200 c.c. This solution was then placed in a collodion bag and dialysed against repeated changes of distilled water, or, since tests showed that the water outside the bag contained no toxin, against running water for 72 hours, using chloroform as an antiseptic inside of the bag. The liquid in the bag was clear, straw-colored and opalescent, it frothed quite readily and dripped fairly viscid. This liquid was centrifuged at high speed to remove some flecks of solid (agar?) and was then poured into four volumes of 95 per cent alcohol. A heavy white precipitate formed at once. The precipitate was allowed to settle in a tall cylinder, the yellowish supernatant liquid was siphoned off and the white solid packed in a tube by centrifugation. The solid was stirred with fresh alcohol, again packed in a centrifuge and dried over sulphuric acid *in vacuo*. Yield 4.85 per cent from the ♀ race and 4.60 per cent from the ♂ race.[6]

Method. (*b*)—One hundred grams of the dried and ground mycelium was stirred into 2000 c.c. of water at the room temperature and allowed to stand with frequent stirring for two hours. The yellowish liquid was then filtered by suction, the residue pressed strongly in a large beef juice press and the liquid obtained added to the first filtrate. Three volumes of 95 per cent alcohol were then added to the united filtrates, yielding at once a copious white precipitate. The partially extracted mould was again stirred into 1000 c.c. of water and allowed to stand at room temperature for 18 hours, when the mixture was filtered by suction and the residue

[1] That a possible slight admixture of the nutrient medium with the mycelium has no significance so far as the toxin obtained is concerned, is shown by check experiments with the fungus grown on oatmeal where all possibility of contamination with the substratum was avoided by carefully cutting off for use only the free aerial mycelium. This preparation showed a toxicity which was *possibly* slightly greater than that of the mycelium from agar.

again pressed dry. This filtrate was likewise precipitated by three volumes of alcohol, yielding a small but appreciable amount of precipitate. The alcoholic solutions were united in a large vessel and allowed to settle for 48 hours, the clear yellow supernatant liquid was then siphoned off and the white solid filtered on a Buchner funnel with suction. The solid was air dried and dissolved as completely as possible in 250 c.c. of water *without the aid of heat.* A considerable quantity did not dissolve (coagulated albumen [?] or agar [?]). The solution was then centrifuged and the clear amber liquid poured into 1000 c.c. of alcohol. The precipitate was allowed to settle, the solution centrifuged and the white solid dried *in vacuo* over sulfuric acid. This solid was powdered and again dissolved in 150 c.c. of water, only a few particles remaining undissolved. The mixture was allowed to stand for 12 hours, centrifuged and poured into 1000 c.c. of alcohol. A white turbidity resulted but on standing for 24 hours no precipitate settled. This was probably caused by an almost complete removal of electrolytes from the solution, for on the addition of two drops of a saturated solution of sodium chloride a copious curdy precipitate was at once thrown down, leaving the supernatant liquid perfectly clear and colorless. This precipitate was packed in a centrifuge, dried *in vacuo* over sulphuric acid and powdered. Yield, 3.58 per cent from the ♀ race and 3.74 per cent from the ♂ race of the mould.

4. *Physiological Tests with the Concentrated Toxin*

(*a*) **The Intravenous Lethal Dose.** — The data for this series can be best expressed in the form of a table, the various preparations of the toxin being designated by (*a*) ♂ (prepared by method [*a*] from the ♂ race of *Rhizopus*) (*a*) ♀, (*b*) ♂, and (*b*) ♀.

(*b*) **Subcutaneous Injection.** — Only one animal was given a subcutaneous injection of the concentrated toxin. Inasmuch as we have found that sterilization with heat causes a loss of toxicity (see "Chemical Properties of the Concentrated Toxin," below), the dry powder was sterilized by placing it in a sterile vial, adding about 5 c.c. of a mixture of ether and 95 per cent alcohol, plugging the vial with sterile cotton and, after allowing the preparation to

Toxin	Wt. injected	Vol. of injection	Wt. rabbit	Time to die	Toxin to body wt.
(*a*) ♂	0.0080 gr.	8 c.c.	1050 gr.	50 mins. "typical"	1: 131,250
	0.0060 gr.	6 c.c.	875 gr.	3 mins. "typical"	1: 145,800
	0.0025 gr.	2.5 c.c.	560 gr.	90 secs. "typical"	1: 224,000
	0.0040 gr.	4 c.c.	1000 gr.	Recovered	1: 250,000
(*a*) ♀	0.0080 gr.	2 c.c.	1000 gr.	19 mins. "typical"	1: 125,000
	0.0040 gr.	1 c.c.	1000 gr.	Recovered	1: 250,000
(*b*) ♂	0.0065 gr.	3.3 c.c.	1390 gr.	5 mins. "typical"	1: 215,000
	0.0050 gr.	2.5 c.c.	1365 gr.	Nearly normal for first 5 hrs. but found dead at 18 hrs.	1: 273,000
(*b*) ♀	0.0080 gr.	4 c.c.	1200 gr.	60 secs. "typical"	1: 150,000
	0.0075 gr.	3.75 c.c.	1510 gr.	75 secs. "typical"	1: 200,000
	0.0072 gr.	3.6 c.c.	1550 gr.	3 mins. "typical"	1: 215,000
	0.0070 gr.	3.5 c.c.	1535 gr.	1 min. 40 secs. "typical"	1: 220,000
	0.0065 gr.	3.25 c.c.	1475 gr.	5 hrs. "typical"	1: 225,000
	0.0036 gr.	1.80 c.c.	810 gr.	Not dead in 7 hrs but dead in 15 hrs.	1: 225,000
	0.0040 gr.	2.0 c.c.	1480 gr.	No typical symptoms. Recovered	1: 370,000

stand for several hours, evaporating the alcohol-ether in a dessicator over sulfuric acid. The dried residue was then dissolved in sterile salt solution.

Under aseptic precautions 0.10 gram of preparation (*b*) ♀, dissolved in 5 c.c. of physiological salt solution, was injected subcutaneously on the back of a rabbit weighing 1550 grams. No immediate effect was observed. Four days later, however, the weight had fallen to 1300 grams and the flesh was very flabby and soft. There was a soft lump low down on the side of the animal and immediately below the point of injection. This lump evidently contained serum or the unabsorbed liquid which was injected. No

sore had as yet opened. The following day, however (five days after the injection) there was a small ulcer at the spot where the lump had been the previous day. The sore continued to spread somewhat, but contrary to the experiments where the "presssaft" was injected, a scab formed over the sore. This scab or thickened skin area could be distinctly felt as a heavy ridge extending from the point of injection to the spot where the skin had broken through. Following the formation of the scab there was a decided improvement in the condition of the animal and 13 days after the injection the weight had risen to 1530 grams. Following this the scab became loosened, leaving the flesh dry. There was no pus formation observed. The animal increased in weight and the lesion healed normally.

(*c*) **Intraperitoneal Injection.** — Two experiments were made in which the concentrated toxin was injected into the peritoneal cavity, using preparation (*b*) ♀.

Rabbit No. 1 weighed 1440 grams and received 1.5 c.c. of a solution containing 0.06 gr. of the toxin, or 1:24,000 parts of body weight. The animal appeared normal for four hours following the injection but was found dead 12 hours later. An autopsy showed that the lower intestine was highly inflamed and that the peritoneal cavity contained a considerable quantity of a yellow fluid, the lungs were discolored with gray blotches but the other organs were normal.

Rabbit No. 2 weighted 1625 grams and received 2.5 c.c. of a solution containing 0.10 gr. of the toxin, or 1:16,250 parts of body weight. No unusual symptoms were observed for some time with the exception that the animal did not seem as active as usual. Forty-eight hours later the weight had fallen to 1450 grams, the temperature was 104°, the animal had diarrhoea and was running at the nose and the skin of the abdomen was highly inflamed. Ninety-six hours after the injection the animal was found dead, weight 1360 grams. An autopsy showed the caecum filled with a highly putrescent liver-colored mass, the stomach packed with food, while the lower intestine and the rectum contained only four or five formed feces whereas normally there is a large number. The small intestine contained a small quantity of a thin yellow fluid (probably bile) and was very much distended with gas. The gall

bladder was three or four times normal size and the liver, otherwise normal, was badly stained with bile. The spleen was much darker than normal, being almost black at the median end. The lungs were a blackish gray, badly mottled, without a trace of pink color being apparent. There was no evidence of inflammation within the body cavity.

(*d*) **Intramuscular Injection.** — Only one experiment was made where the injection was given intramuscularly, and this injection was not an intentional one. A rabbit weighing 1310 grams was given an injection of 5 c.c. of a solution containing 0.20 grams of preparation (*b*) ♀. The intention was to give the injection intraperitoneally, but later developments and the autopsy showed that the needle had not penetrated the abdominal wall and that the injection had been between the muscle plates of the abdomen. Forty-eight hours after the injection the weight had fallen to 1180 grams, the temperature was 104.9°, the skin of the abdomen was highly inflamed and very tender to the touch, but no sores were observed and the animal ate regularly. Eight days after the injection the weight had fallen to 1045 grams and a bad purulent ulcer had formed on the abdomen. The animal was chloroformed and an autopsy performed. All of the viscera were in a normal condition and only a slight inflammation was observed on the inner wall of the abdomen immediately under the ulcer. In this instance, therefore, an intramuscular injection caused the same developments as a subcutaneous injection, only the symptoms were somewhat more pronounced. This may be due to the higher dosage of toxin (30 to 40 intravenous lethal doses) employed, or it may be due to the fact that the irritation was between the muscle plates rather than subcutaneous.

(*e*) **Administration of the Concentrated Toxin per Os.** — Only one feeding experiment was made but the quantity of the toxin was sufficiently large to determine whether or not the mould would be dangerous in moderate doses.

A rabbit weight 1330 grams was fed, by means of a catheter inserted into the stomach, 50 c.c. of a solution containing 1.0 grams of preparation (*b*) ♂ — in other words 150 to 200 times the intravenous lethal dose. *This is the equivalent of 26 to 27 grams of the dried mould mycelium and of probably 100 to 125 grams of the*

moist growing mould. It is a far larger amount than any animal could possibly procure at any one feeding under any natural conditions. No abnormal effect has been observed to date. The weight of the animal steadily increased, the animal weighing 1590 grams 19 days after the feeding.

From this experiment, in which we are certain that no loss of the toxin occurred through vomiting, we conclude that, in rabbits at least, the toxin is not absorbed from the alimentary canal, or if absorbed it has been so changed as to produce no ill effects.

5. *Chemical Properties of the Concentrated Toxin*

The product obtained by either method (*a*) or (*b*) forms a yellowish white powder readily soluble in water to a straw-colored opalescent solution from which it is again precipitated by basic lead acetate, phosphotungstic acid + HCl (without the addition of the HCl only a white opalescence is produced), three or four volumes of alcohol, and by mercuric chloride. Tannic acid gives a fine white precipitate with a solution of the toxin, and Folin's "Phenol Reagent" gives a deep indigo-blue color.

The Biuret, Xanthoprotein, Adamkiewicz's, Liebermann's and Molisch's tests are all strongly positive, while Millon's test is positive but faint. The substance does not reduce Fehling's solution, but after hydrolysis with acids a strong reduction takes place. The toxicity of the solution is not altered by passing through a Berkefelt filter, but on warming with freshly ignited animal charcoal all of the toxin is absorbed by the charcoal and the solution is absolutely non-toxic. This is still the case when the solution + bone black is quickly raised to *just boiling* and then rapidly cooled.

Digestion at 37° with pepsin in 0.2 per cent HCl for three hours did not impair the toxicity, but a further digestion of this solution with trypsin in a weakly alkaline medium for 18 hours (no antiseptic) caused a loss of about 75 per cent of its activity.

The toxicity is slowly destroyed by boiling. 0.20 grams of preparation (*b*) ♂ was dissolved in 50 c.c. of physiological salt solution and boiled under a reflux condenser for three hours. There was no evidence of decomposition nor was there any precipitate formed, but on testing the physiological activity of the solution it was found that the lethal dose was about 1:37,000 parts of body

weight. This would indicate the loss of about 75 per cent of the original activity.

When, however, the toxin is boiled with N/10 HCl there is a rapid loss of activity. 0.20 grams of preparation (*b*) ♂, was boiled under a reflux condenser with 25 c.c. of N/10 HCl for $1\frac{1}{4}$ hours, 25 c.c. of N/10 NaOH was then added and the solution, which now gave a strong reduction with Fehling's solution, was poured into four volumes of 95 per cent alcohol. The white precipitate which was thrown down was collected in a centrifuge, dried, dissolved in a small volume of physiological salt solution and injected into a rabbit intravenously. The dosage was equivalent to 1:14,000 but no ill effects were observed, indicating that all or nearly all of the toxin had been destroyed.

The possibility that we were dealing with a saponine was tested by adding a strong solution of the toxin to washed blood corpuscles, but no trace of hemolysis occurred.

Analyses

Nitrogen was determined on the different preparations in part by Dumas' method and in part by Kjeldahl's. From the nitrogen figures it is seen that we are not dealing with a pure compound but with a mixture, and therefore, no further analyses were attempted. From the figures for nitrogen and from the chemical tests to which the toxin responds one can postulate all sorts of classifications, such as glucoside, the split product of a protein, peptides, etc., but we are unwilling to even venture a guess as to the chemical nature of the toxin. That can only be determined when some method has been devised by which the toxic principle can be obtained in a pure condition.

Preparation (*a*) ♂:
0.1916 gr. gave 7.4 c.c. N at 24° and 768 mm. Nitrogen found = 4.43%.

Preparation (*a*) ♀:
0.2300 gr. gave 5.4 c.c. N at 22° and 768 mm., 0.4180 gr. gave 9.0 c.c. N/10 NH_4OH. Nitrogen found = 2.69% and 3.01%.

Preparation (*b*) ♂:
0.2554 gr. gave 10.9 c.c. N at 20.5° and 762 mm., 0.2260 gr. gave 9.6 c.c. N at 21.5° and 768 mm. Nitrogen found = 4.88% and 4.87%.

Preparation (*b*) ♀:
0.2977 gr. gave 10.25 c.c. N/10 NH_4OH, 0.2253 gr. gave 9.0 c.c. N at 23° and 767 mm. Nitrogen found = 4.82% and 4.54%.

6. *Evidences of a Toxin in Other Species of the* Mucorineae

During a series of injections we have obtained some evidence that there may be a small amount of a toxic substance in the "presssaft" of other moulds, such as "*Mucor V*" and *Phycomyces nitens*. We have made a large number of intravenous injections of the "presssaft" of these species and have observed that where a non-lethal dose is increased in an injection three to four days later death may occur. In two sets of experiments we have lost four out of five rabbits injected with the ♀ race of *Mucor V* while none of the animals receiving an equal quantity of the "presssaft" from the ♂ race have died. It was also observed that whereas 2 c.c. of the "presssaft" from the filaments of *Phycomyces* caused no untoward symptoms, that an injection of 3 c.c often caused convulsions and sometimes death. An attempt was therefore made to concentrate the toxin from *Phycomyces*, if such a toxin existed.

Twenty grams of the dry aerial filaments of the ♂ race of *Phycomyces nitens* was treated as in method (*a*) (page 358). The alcohol precipitate was dark green and the powder on drying was almost black. A solution of the dried preparation was too dark to permit of the observation of color tests. A solution of the powder did not reduce Fehling's solution, but after acid hydrolysis a strong reduction was observed. A yield of 2.60 per cent of the dried product was obtained.

In physiological tests no effect was observed when an intravenous injection of 1:15,000 was given. This preparation has therefor little or no toxic properties.

The fact that *Phycomyces* gives a yield of 2.60 per cent of a non-toxic substance by the same method that *Rhizopus* yields 4.60 and 4.85 per cent of a toxic substance confirms to our mind the fact that our toxin is far from being a pure compound. By the method of preparation glycogen, or glyco-proteins, would, if present, be precipitated with the toxin and would account for the Molisch's and Fehling's tests.

Summary

1. *Rhizopus nigricans* contains a powerful toxin which is water soluble and which can be completely precipitated from its aqueous solution by three to four volumes of 95 per cent alcohol.

2. A solution of the toxin is non-dialyzable, gives the protein tests as well as Molisch's test, but reduces Fehling's solution only after a previous hydrolysis with acid. The toxin is completely absorbed from its aqueous solution by warming with bone black, is slowly destroyed by boiling its aqueous solution and rapidly destroyed by boiling with N/10 HCl. Peptic digestion for three hours is without effect on the toxicity.

3. In intravenous injection into rabbits the lethal dose of our preparations lies between 1:225,000 and 1:275,000 parts of body weight.

4. In subcutaneous and intramuscular injections the toxin causes the formation of purulent ulcers.

5. In intraperitoneal injections there is some evidence that paralysis of the digestive tract ensues.

6. Administered *per os* in large doses there was no harmful effect observed.

7. Inasmuch as a 2.6 per cent yield of a *non-toxic* substance having similar chemical reactions can be prepared from *Phycomyces nitens*, and inasmuch as the nitrogen content of our toxic preparations varies from 2.8 per cent to 4.9 per cent, we believe that our preparations are, in all probability, highly impure, perhaps containing 50 per cent or more of inactive impurities, so that the actual intravenous lethal dose of the pure toxin is probably much higher than has been observed, possibly near the extreme toxicity of 1:500,000.

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EXPERIMENTS ON THE ORIGIN AND PROPAGATION OF THE IMPULSE IN THE HEART

IV. The Effect of Vagal Stimulation and of Cooling on the Location of the Pacemaker within the Sino-auricular Node.[1]

By WALTER J. MEEK and J. A. E. EYSTER

[*From the Physiological Laboratory of the University of Wisconsin*]

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IN a recent paper[2] we have shown by using the method of initial electric negativity to locate the origin of the heart beat, that stimulation of the vagus nerve might remove the seat of impulse formation from one part of the mammalian heart to another. So far as could be determined by means of initial negativity the pacemaking function always remained in some portion of the specialized tissue. Not only might the pacemaker migrate from the sinus region to the auriculo-ventricular node, but as first demonstrated by Zahn[3] it might be restricted in its location to certain parts of the latter. This we confirmed, and in one case we were able to convert a coronary sinus rhythm into an auriculo-ventricular one by vagal stimulation.

The discovery that the auriculo-ventricular node need not function as a whole but that a part may act as the seat of impulse formation suggested at once that the sino-auricular node be investigated along similar lines. Such an investigation, however, was brought to mind not only by this previous work on auriculo-ventricular rhythm but also by certain curves we had obtained on comparing various parts of the sulcus terminalis with each other.

[1] The preceding papers of this series have appeared in Heart, 1914, v, Nos. 2 and 3.

[2] Meek and Eyster: Heart, 1914, v, No. 3.

[3] Zahn: Archiv für die gesammte Physiologie, 1913, cli, p. 247.

Usually an electrode could be placed on a point which histological examinations had taught us was near the head of the node with assurance that this point would precede all others along the sulcus terminalis in negativity. At times, however, the area of initial negativity was lower than expected. A possible explanation was that the node was not functioning as a whole but that in these cases the lower part was acting as pacemaker. Experiments testing this idea seemed of value not only of themselves but for the light they might throw on the mechanism of vagus action and on the gradual shortening of the As-Vs interval which is often observed during the appearance and disappearance of auriculoventricular rhythm.

There is no experimental evidence which shows how small an amount of automatic tissue may function as pacemaker. The sinus node is, however, of considerable size and a division into functional parts seems quite possible. According to careful measurements by Lewis, Oppenheimer and Oppenheimer [1] the sinus node in 7 dogs averaged 13.7 mm. in length and 2 mm. in width. Koch [2] found the node 7 mm. long in a rabbit's heart. In 4 dogs' hearts examined histologically by ourselves the nodes averaged a trifle over 15 mm. in length. The sinus node is then of sufficient size to allow an analysis into parts by the electrical method of initial negativity.

Experimental Methods

In our first series of experiments the upper, middle and lower parts of the sinus node were compared with each other by means of the string galvanometer before, during and after periods of vagal stimulation. Non-polarizable electrodes were used which were attached to the heart with pieces of woollen yarn, the latter being stitched to the epicardium by a fine thread. This means of attachment insured a constant contact during all parts of the cardiac cycle. With suitable keys any of the points to be studied could be connected through the galvanometer and a photographic record of the movement of the string made on bromide paper with a long roll photographing apparatus. Precedence in activity

[1] Lewis, Oppenheimer and Oppenheimer: Heart, 1910–11, ii, p. 147.

[2] Koch: Medizinische Klinik, 1912, viii, p. 108.

was then determined by the direction of the auricular wave. To identify this wave the mechanical contraction of the auricle was also recorded by air transmission to a Marey tambour.

Although these experiments were positive, showing that during vagal stimulation the lower part of the sinus node might precede the upper in activity, it was felt that the results were not conclusive evidence of the shift of the pacemaker within the sinus node itself, since the beats at this time might have been arising in the auriculo-ventricular node, a condition which might show the lower part of the sinus node negative before the upper. To meet this objection it seemed necessary to compare the upper part of the sinus node with the lower, and the sinus node with the auriculo-ventricular node simultaneously. With the aid of two galvanometers this has now been done and it is these experiments that we wish to report at this time.

As mentioned before, three non-polarizable electrodes were placed as well as could be judged on the upper, middle and lower parts of the sinus node. To reach the auriculo-ventricular node a long curved glass electrode was passed down the external jugular vein and its end adjusted against the auricular septum just above the middle tricuspid valve. The middle sinus and auriculo-ventricular electrodes were now connected through the first galvanometer and the upper and lower sinus electrodes through the second. The electrodes were so connected that upstroke on the photographic curve in each case represented primary activity of the first member of the couple compared. In order to identify positively the auricular wave the mechanical contraction of the right auricle was recorded by means of air transmission. A signal to show the beginning and end of procedures and time in one-fifth seconds were also registered on the records. All the above were recorded on a single record, bromide paper of 12 cm. width being employed for this purpose.

Dogs were used in all experiments. The animals were morphinized, the chest opened and the heart exposed under ether anesthesia. The excellent artificial respiration apparatus recently described by Gesell and Erlanger[1] was used. To this was added an electric heating coil which was thrown into circuit by a thermo-

[1] Gesell and Erlanger: This journal, 1914, xxxiii, p. 33.

regulator and relay. In this way the air delivered from the tank was maintained at 55° C. and the mixture leaving the ether bottle was kept at approximately 37° C. With this slight modification of the Gesell-Erlanger apparatus the difficulties and inconveniences of artificial respiration seem almost entirely overcome.

The hearts were removed after the experiment and preserved in formalin. The position of the auriculo-ventricular electrode was carefully noted and in the first experiments histological examinations of the sinus region were made. In all hearts examined the position of the electrodes was either on or near enough the part desired to justify our conclusions. In the latter experiments the hearts have not been studied histologically but the electrodes were placed with extreme care and judging from the fairly constant position of the sinus node we feel certain they were either on or near the ends of the sinus node.

The vagi were stimulated with tetanizing currents from a Harvard induction coil. The most desirable strength of stimulus was found to be one that only slightly showed the heart. Pencils of ice and ethyl chloride sprays were used to cool the sinus node.

Experimental Results

1. *Vagal Stimulations.* — Three experiments were carried out in which comparisons of the upper sinus region with the lower, and of the sinus node with the auriculo-ventricular node, were made simultaneously during vagal stimulation. Thirty records were taken in 19 of which the right vagus was stimulated and in 11 the left. The following are the most important results obtained by a study of these records.

In 10 of the 30 records instances were found either of single beats or series of beats in which the upper curve comparing sinus and auriculo-ventricular node remained unaltered in direction, while the lower curve comparing the upper and lower parts of the sinus node had reversed. Fig. 1 illustrates a case of this kind. In the first two cycles of this record the auricular wave of each curve begins with an upstroke, indicating that the sinus node preceded the auriculo-ventricular node (upper curve) and that the upper part of the sinus node was active before the lower

(lower curve). In the third cycle at the end of a two-second period of left vagal stimulation the upper curve remains the same in direction, but the auricular wave of the lower curve has reversed. This indicates that while primary activity was still in the sinus

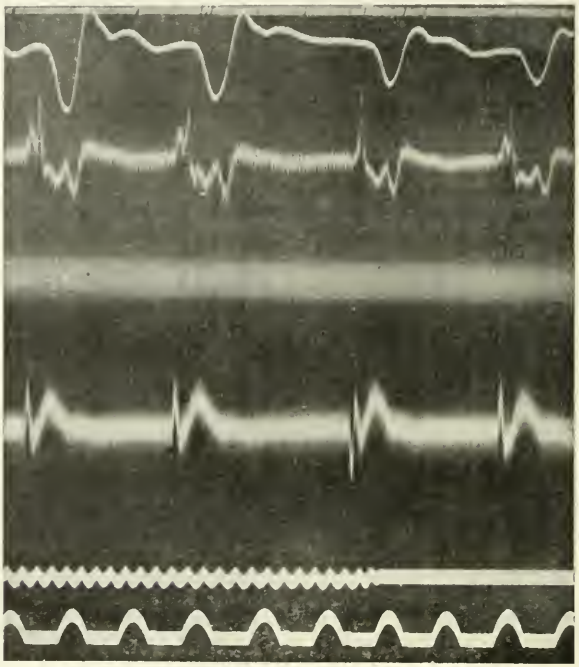

FIGURE 1. Simultaneous comparisons of the sinus node with the auriculo-ventricular node (upper curve) and of the upper part of the sinus node with the lower (lower curve). The third cycle just at the end of vagal stimulation shows a beat arising in the lower part of the sinus node as indicated by the reversal in direction of the auricular wave in the lower galvanometer record.

In all figures the uppermost curve is from a tambour showing the mechanical contraction of the auricle, the second curve is from the string of the upper galvanometer, the third curve is from the string of the lower or second galvanometer, the fourth curve is from a signal showing the duration of vagal stimulation or cooling, and the fifth curve is from a clock marking fifth seconds.

region as shown by the upper curve, the point of initial activity, as shown by the reversal in the lower curve, had shifted from the upper to the lower part of the sinus node. In this case the condition did not persist, for the fourth cycle shows a return of the curves to their original forms.

In Fig. 2 is reproduced a portion of a record showing the

return to normal after a left vagal stimulation which had lasted three seconds. A reversal of the lower curves was produced which consisted of 14 beats and outlasted the stimulation two and one half seconds. The last two cycles of this series are the first two showing in the figure. The return of the pacemaker to the upper

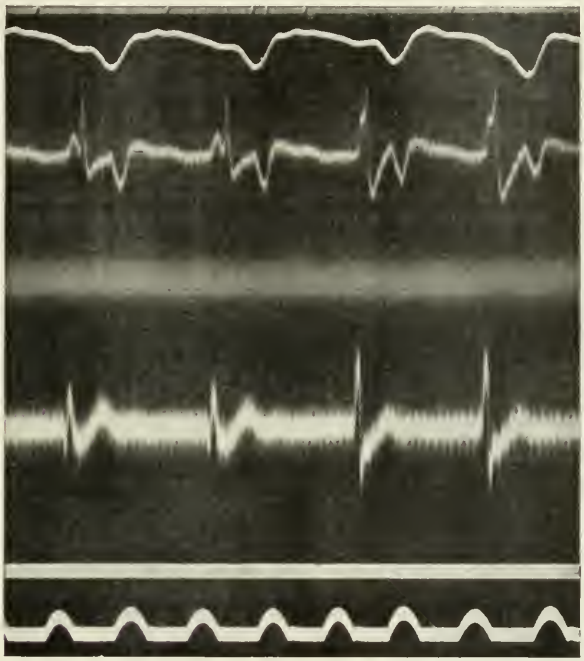

FIGURE 2. Comparisons the same as in Fig. 1. Impulse formation was shifted to the lower part of the sinus by vagal stimulation. The reversal of the auricular wave in the third cycle of the lower galvanometer shows the return to normal.

part of the sinus node is made evident by the third and fourth cycles of the lower curve beginning with an upstroke. It is such examples as these which have led us to conclude that vagal stimulation may depress only a part of the sinus node, allowing another part to take up the pacemaking function.

The ultimate automaticity of a part is determined by its rate of discharge. If according to the hypothesis of vagal action which will be presented later in this paper, a region of high automaticity, the upper part of the sinus node, has been depressed by vagal stimulation, and a region of less automaticity, the lower part of the

sinus node, has taken on the pacemaking function, then the heart rate should be slower. This idea is amply borne out by our records. The second cycle in Fig. 1 is about .08 sec. longer than the average. In Fig. 2 the cycles showing reversal of the lower curve are .03 sec. longer than the normal.

Although most of the cycles occurring with the pacemaker in the lower part of the sinus node have shown shortened As-Vs intervals as may be seen in the third cycle of Fig. 1, we have not felt like drawing conclusions from such results since the As-Vs interval may be greatly modified by the dromotropic influence of the vagus. Such data we believe will be much more valuable from experiments in which the sinus node is depressed in some way, as by localized cooling, which would not produce a widespread influence on conduction.

In several records we have observed during vagal stimulation a splitting of the auricular wave in the sinus node — auriculo-ventricular node lead. Examples of this may be seen in Figs. 1 and 2, the condition disappearing in the latter record as vagal influence ceased and the pacemaker returned to the upper part of the sinus node. We have previously shown [1] that a sino-auricular interval exists amounting to about .025 sec. In these curves it seems that the sino-auricular conduction was so depressed by vagal action that the contraction of sinus as well as auricle produced its effect on the galvanometer. This occurrence we have noted in previous experiments and have discussed its possible significance in a former paper.[1] The lower record does not show the two waves since here the two electrodes were each on sinus tissue and the contraction of the auricle produced little or no effect.

In our experiments the left vagus was much more effective in shifting the pacemaker to the lower part of the sinus node than the right. In only two cases did we secure results from the right vagus. This at first seems somewhat contradictory to the recent work which is in favor of a relative homo-lateral distribution of the vagi, but on closer analysis our results rather support such conclusions. Removal of the pacemaker from one part of the sinus node to another can only be brought about by weak vagal stimulation. This is evident from the absence of extra systoles,

[1] Eyster and Meek: Archives of internal medicine, 1913, xi, p. 204.

reversed cycles and block in all our successful records. This proper degree of stimulation may have been easiest obtained with the left vagus for the very reason that it sends fewer fibers to the sinus node. Our stimulations of the right vagus were usually strong enough to produce extra systoles and other irregularities and consequently fewer cycles with the pacemaker removed merely to the lower part of the sinus mode.

2. *Cooling the Upper Part of the Sinus Node with Ice and Ethyl Chloride.* — It seemed to us that if it was the depressant action of the vagus on the upper part of the sinus node which allowed the lower part of the node to express its automaticity by assuming the pacemaking function, then any procedure which would depress the upper part of the node ought to give similar results. Experiments were therefore planned in which the upper part of the sulcus terminalis was cooled with ice or ethyl chloride sprays. Seven such experiments were carried out in each of which long series of reversals were obtained indicating that the pacemaker had shifted to a lower part of the sinus node.

In Figs. 3 and 4 may be seen the removal of the pacemaker to the lower part of the sinus node and its return as portrayed by the electrical curves. A pencil of ice was applied to the upper part of the sulcus terminalis two seconds before the beginning of Fig. 3. The sixth cycle of the figure shows a reversal of the auricular wave in the lower curve. Between Fig. 3 and Fig. 4 a space amounting to four and one half seconds of the record and including 10 cycles with reversed auricular waves has been omitted. In the fourth cycle of Fig. 4 the auricular wave of the lower curve begins with an upward stroke, showing the return of initial activity to the upper part of the sinus node.

This shifting in the point of negativity we have secured repeatedly in each of the seven experiments by cooling either with ice or ethyl chloride. That the response was due to some specific effect on the upper part of the node was proved in each experiment by cooling the lower part of the sulcus terminalis. This procedure was invariably ineffective. On freezing the entire sinus node with ethyl chloride auriculo-ventricular rhythm appeared.

In these experiments as in the previous ones concerned with vagal stimulation, the descent of the pacemaker to a lower part of

the sinus node was always marked by a lengthened cardiac cycle. This is best seen in Fig. 4 where the shortening of the cycles as the pacemaker returns is easily noted. The length of cycle during the time in which the auricular wave was reversed equalled .458 sec. This shortened down to .411 sec. as the pacemaker migrated upward.

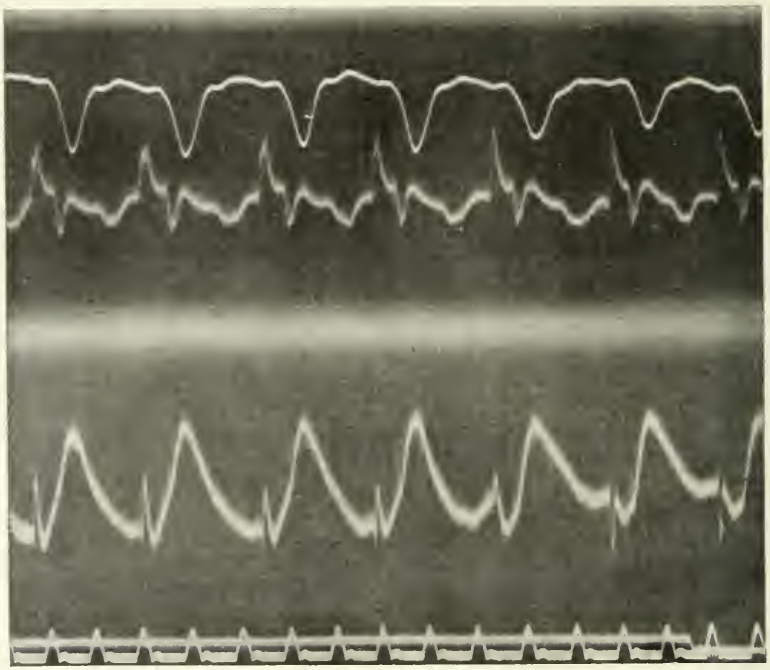

FIGURE 3. Effect of cooling the upper part of the sinus node for three seconds with a pencil of ice. The reduction and final reversal of the auricular wave in the lower galvanometer curve indicates the assumption of the pacemaking function by the lower part of the sinus node.

Another point of great interest was the shortening of the As-Vs interval which occurred as the pacemaker moved downward. This may be most clearly seen in Fig. 3. The As-Vs interval of the first two cycles equals .102 sec. while in the last cycle of the figure the interval has become reduced to .091 sec. This reduction though slight has been constant. Its significance will be discussed in the next section.

3. *Injection of KCl.* — On the basis of Howell's[1] theory that vagal inhibition is due to a liberation of K ions in the automatic tissues it seemed that a migration of the pacemaker from the upper part of the sinus node might be expected if an amount of KCl just sufficient to depress this part could be injected into the blood stream. This was accordingly tried in three experiments

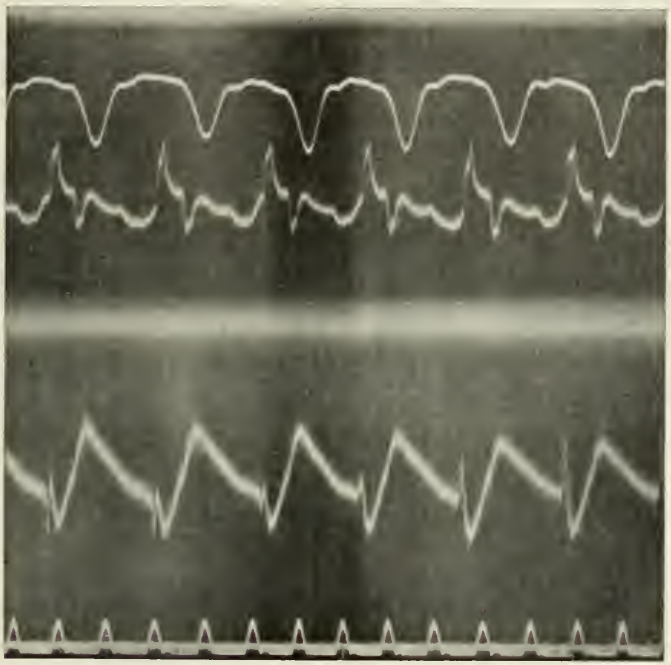

FIGURE 4. This is a continuation of Fig. 3 taken four and one half seconds later. As the effect of the cooling wears off the auricular wave of the lower galvanometer reverses, indicating the resumption of the pacemaking function by the head of the sinus node. Throughout all records the direction of the auricular wave in the upper galvanometer has remained unchanged, showing that at all times the seat of primary activity was in the sinus region.

and in a fourth the sulcus terminalis was painted with 5 and 10 per cent KCl solution.

In two of the experiments following injections of KCl there was a reversal of the lower curve showing that the pacemaker had left the upper part of the sinus node. In the first of these cases the condition was brought on by an intravenous injection of 6 c.c.

[1] HOWELL and DUKE: This journal, 1908, xxi, p. 51.

of a 5 per cent KCl solution in $1\frac{1}{2}$ minutes. This had, however, been preceded shortly before by an injection of 4 c.c. In this experiment the tracings did not return to normal and investigation showed that a coronary sinus rhythm had been produced. This might be readily explained on the basis that the dose of KCl was large enough to paralyze the entire sinus node. In another experiment following an injection of 10 c.c. of a 5 per cent KCl solution in $1\frac{1}{2}$ minutes there was a shifting of the pacemaker from the upper to the lower part of the sinus node as shown by reversal of the lower curve and the absence of any change in the upper. The curves later returned to normal.

Although these experiments are few in number they show that in KCl we have another means of depressing the upper part of the sinus node with the assumption of impulse formation in some lower part. This elective depressant action would seem to lend support to Howell's theory of vagus inhibition.

Discussion

Most of the recent physiological work has emphasized the part played by the specialized tissue of the heart in the initiation and conduction of excitation. Our own work has shown that with the heart in situ beats arising outside of the specialized tissue are extremely infrequent, if they occur at all. By electrical methods we have found in agreement with Ganter and Zahn [1] and Zahn [2] that if the sinus node as a whole is destroyed, depressed or isolated some lower part of the specialized tissue at once takes on the pacemaking function. Strong vagal stimulation was found to be an effective means of depressing the entire sinus node and producing auriculo-ventricular beats. In the present paper we have shown that with weak vagal stimulation or other means of moderate depression such as local cold, the seat of impulse formation may migrate from the upper to the lower part of the sinus node.

Following the principle long ago laid down by Gaskell and Engelmann and so often insisted on by Hering, that the most

[1] Ganter and Zahn: Archiv für die gesammte Physiologie, 1912, cxlv, p. 335.

[2] Zahn: Archiv für die gesammte Physiologie, 1913, cli, p. 247.

automatic part of the heart is the pacemaker at that given moment, our experiments detailed above have led us to the following conception of the action of the specialized tissue and the vagal mechanism of the heart. The specialized tissue of the heart exhibits from above downward progressively diminishing degrees of automaticity. When the dominant rhythm of the heart arises from the highest parts of this system the rate is maximal, other conditions remaining constant, and when it arises from the lowest parts the rate is minimal. Intermediate rates result from some part between these assuming the rôle of pacemaker. Each part of the specialized tissue has of course its own maximum and minimum rate, the exact rate at which it functions at any time depending on nervous influences, temperature and other factors affecting it. It is this maximum which is highest for the upper part of the specialized tissue, that is the sinus node, and which decreases progressively downward.

Those fibers of the vagus that influence the rate of the heart, the chronotropic fibers, are distributed to the specialized tissue comprising the sino-auricular and auriculo-ventricular nodes and their branches. The specific function of the chronotropic fibers of the vagus is to depress automaticity in the specialized tissue. The mass of innervation, that is to say the number of fibers distributed to any region, is an important factor in determining the amount of effect produced on this region when the vagus trunk is stimulated. With weak stimulation of the vagus only those regions would be affected which receive a proportionately large number of fibers. With stronger stimulation the effect might spread to other regions of specialized tissue receiving a less profuse chronotropic innervation. The greater number of vagus chronotropic fibers are distributed to the most automatic part of the sino-auricular node, that part which normally acts as pacemaker for the whole heart. Other parts of the sinus node, with a smaller degree of inherent rhythmicity, receive relatively fewer chronotropic fibers. The auriculo-ventricular node and its connections, representing that part of the specialized system which has a relatively lower degree of automaticity, receives a still smaller number of chronotropic vagus fibers.

Light vagal stimulation, as in stimulation of the vagus trunk

with a weak electrical current, will affect to an appreciable degree only that part of the sino-auricular node which possesses the highest degree of automaticity. The automaticity of this part will be depressed until it is lower than that of some other portion of the node and the latter will at once assume the control of the heart rhythm, or in other words, become the pacemaker. The net result is a slowing of the whole heart.

To give a concrete example one may suppose that there are two points within the sino-auricular node, *A* and *B*, which have different degrees of automaticity, such that *A* is able to excite excitations at the rate of say 100 to 70 beats per minute, while *B* can initiate impulses as a result of its inherent automaticity only at rates between say 80 and 50 per minute. Unless there is still a third region which has a higher rate of discharge, *A* will dominate all other regions and will act as pacemaker for the whole heart at a rate somewhere between 100 and 80. If now the vagus is stimulated, and if this nerve due to more profuse distribution of its fibers to *A* has a greater influence on this region than on *B*, then the automaticity of *A* may be so reduced that its power to discharge impulses will fall below that of *B*, say to 70 or 75 per minute, *B* not being markedly depressed since it has a less profuse vagal innervation, will now be the most automatic part of the heart and will at once assume the rôle of pacemaker for the whole heart. Change in rate is due first to depression of *A* and second to a change in the seat of impulse formation from a point of higher to lower automaticity.

Still stronger stimulation of the vagus may now depress the power of *B* and the pacemaking function will then be assumed by a third region of still lower automaticity. In this way it may be understood how a stronger and stronger stimulus may cause a progressive reduction in heart rate accompanied by a migration downwards of the pacemaker. If finally the stimulation of the vagus becomes sufficiently strong so that even the lower parts of the specialized tissue with their poor innervation are depressed, all impulse formation may cease and we have for a time a complete vagal inhibition of the heart.

We are aware that there have been intimations of some such conception of the automatic tissues of the heart and of vagal action

as that presented above, but we do not believe it has been previously presented in a complete form and certainly it has never been supported by the physiological evidence now at hand. This theory of the specialized tissues in the heart and of vagal action has support in and has been deduced from the following experimental facts.

1. As shown in the present paper slight degrees of vagal stimulation may cause the pacemaker to migrate from the upper to the lower part of the sinus node. This change is always accompanied by a slight slowing in rate.

2. Other depressing agents such as ice and ethyl chloride when applied to the upper part of the node cause the seat of impulse formation to remove to lower parts of the node.

3. If the sinus node be subjected to extremes of vagal stimulation or cooling with ice and ethyl chloride, or if the node be injured, destroyed, or isolated by crushing, cutting or the application of drugs, the pacemaker of the heart migrates temporarily or permanently to lower parts of the specialized tissue, usually the auriculo-ventricular node.

4. Flack [1] has shown that there is a profuse supply of chronotropic fibers to the sino-auricular node, greater than to other parts of the heart.

5. In auriculo-ventricular rhythm, in which the pacemaker resides in the auriculo-ventricular node, the chronotropic action of the vagus is very much reduced.

The shortening of the As-Vs interval observed in our experiments when the lower part of the sinus node became pacemaker seems to us of considerable interest. The gradual shortening of this interval, sometimes seen as auriculo-ventricular rhythm appears or disappears, has always been a difficult thing to understand. We have recently ventured to suggest that such variations of the As-Vs interval were in large measure associated with a shifting of the physical location of the pacemaker. The data now at hand seems to substantiate that view.

We have previously shown [2] that conduction from the sinus

[1] Flack: Journal of physiology, 1910–11, xli, p. 64.

[2] Eyster and Meek: Heart, 1914, v, p. 119.

node to the auriculo-ventricular node cannot be by way of the auricle. The path to the auricle is probably a diffuse one directly across the sulcus terminalis while the path to the auriculo-ventricular node is a linear one, just how well circumscribed we are not able at present to say. As the seat of impulse formation passes downward the time of access to the ventricle shortens and that to the auricle remains the same or lengthens. Since it is now known that the lower part of the sinus node and the auricular portion of the auriculo-ventricular node may act as pacemaker, the gradual shortening of the As-Vs interval may well be explained in large part at least by the migration of the pacemaker through these regions.

On the hypothesis that the specialized tissue of the heart is the seat of all automatism and conduction, then any point to which the pacemaker is forced should lie in this system. The migration of the seat of impulse formation from the head of the sinus node first to a lower part of the node thus becomes very suggestive. If there is a definite path between the sinus node and the auriculo-ventricular node, then this new seat of impulse formation should be in it. If there is a special path from the higher auricular parts to the lower, it seems that it must pass through the lower part of the sulcus terminalis. If the connection between the sinus node and the auriculo-ventricular node is diffuse, then the migration of the pacemaker to the lower part of the sinus node has no interest so far as a circumscribed path of conduction is concerned.

Summary

With the aid of two string galvanometers, one comparing the upper with the lower part of the sinus node and the other comparing the sinus node with the auriculo-ventricular node, it has been shown that by means of vagal stimulation, cooling of the upper part of the sinus node or injection of potassium chloride, the point of initial negativity may be made to shift from the upper to the lower part of the sinus node. This has been interpreted as showing that during these procedures the pacemaker of the dog's heart may move from the upper to the lower part of the sinus node.

During the time that the seat of impulse formation resides in a lower part of the sinus node there is a lengthening of the cardiac cycle and a shortening of the As-Vs interval.

On the basis of work presented in this paper and others of the series, a theory has been presented which correlates our experimental results on the automatic and vagal mechanisms of the vertebrate heart. It is believed that the specialized tissues of the heart exhibit from above downward progressively diminishing degrees of automaticity. Vagal chronotropic innervation of the specialized tissue also diminishes from above downwards. The most automatic portion of the specialized tissue acts as pacemaker for the heart. The function of the chronotropic fibers is to depress this automaticity. When the automaticity of the pacemaker is reduced below that of a lower part the latter assumes dominance and becomes pacemaker. In this way the vagus, if the stimuli are properly graded, may cause the pacemaker to descend from the upper part of the sinus node where it resides normally, to the lower part of the sinus node, and finally even to the auriculo-ventricular node.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 249

REACTIONS OF EARTHWORMS TO HYDROXYL IONS

By A. T. SHOHL

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I. Introduction

Parker and Metcalf ('06) demonstrated the effect of salt solutions, and Hurwitz ('10) of acids, on earthworms. At Prof. G. H. Parker's suggestion I attempted to determine the effect of hydroxides on earthworms.

The worms were immersed in solutions of hydroxides. They withdrew from it when possible. This phenomenon can be regarded as a physiological reaction to a stimulus; there are nerves in the epidermis whose stimulation results in the contraction of muscles. The animal can also be considered as one whose cellular tissue is composed of proteins in colloidal solution. The environment can be regarded as solutions which are dissociated into positive basic ions and negative hydroxyl ions. It is this latter point of view that I adopt for the purposes of the present work. The problem then becomes the mechanism of protoplasmic stimulation by electrolytes.

Under the conditions of the experiment the worms withdrew from all solutions. The different ions effect the specific nerve endings, quantitatively not qualitatively. The hydroxides of sodium and potassium were compared as to their relative intensity as a source of stimulation; the hydroxyl ions and basic ions were compared as to which was the greater factor in producing the effect. Several of the factors involved were investigated, — namely: a non-stimulating solution in which to keep the worms, the effect of distilled water, and that of temperature. In order to improve the conditions of the experiment a new method was devised for submitting the worms to the solution.

The results obtained in the investigation were subjected to an analysis of the physicochemical factors involved. The conclusion derived is that stimulation is dependent on the specific nature of the ion, probably through the electrical action it produces.

II. Method

The method first used was that described by Parker and Metcalf ('06, p. 56). The worms tested in these experiments were Allolobophora foetida. They were obtained from a large manure heap in Cambridge where they could be found all through the winter. At first they were collected fresh every few days. Later this was found unnecessary, for they could be kept for an indefinite time in the laboratory in jars containing manure.

A worm was prepared for the test by being rinsed in a little tap water to remove the dirt. Through the posterior region a silk thread was passed. The worm was then placed in a small, wide-mouthed jar which contained filter paper and a little water. This was so inclined that the worm could crawl in or out of the water. When ready for use the worm was removed from the jar by means of the thread, care being used not to let it touch the sides. It was suspended on the end of a lever arm like a bucket in a well. When it had become extended, it was lowered into the solution to be tested, as far as the anterior edge of the clitellum. As it touched the solution a stop-watch was started and when it withdrew from the solution the watch was stopped. The worm was then taken off, dipped in tap water, and returned to its jar. The time of immersion was recorded and called the reaction-time. The solutions were taken so that each worm was used first in some one solution and then was dipped in every other solution before it was again exposed to the first solution. The object of this procedure was to prevent the worm from being exposed exclusively to one solution, and hence to permit direct comparisons. Every worm was first in some one solution. This was meant to eradicate the possibility of the first immersion influencing the subsequent trials. Averages of eight or more readings were taken to obviate the error of individual results.

The solutions used were made up from the purest obtainable

sodium and potassium hydroxide sticks (Merck). The stock solutions, from which the others were made as needed, were in semi-normal concentrations. These were titrated against semi-normal oxalic acid with phenolphthalein and methyl-orange as indicators. The former registers only the hydroxyl ions; the latter, total alkalinity.

	Phenolphthalein:	Methyl-orange:
NaOH	$.97 \frac{m}{2}$	$1.28 \frac{m}{2}$
KOH	$.85 \frac{m}{2}$	$1.21 \frac{m}{2}$

III. Some Factors in the Reaction

Variation in Reaction Time. Even when all the conditions are as nearly uniform as can be obtained, there is a great difference in the reaction of these animals. Even with the same solution on the same day the individual differences may amount to over one hundred per cent in the duration of the reaction. Not only is this true, but as Towle ('04) has found for paramoecium also, animals change in reaction from day to day and with the seasons. The condition of breeding may also be a factor, according to Towle ('04). The size of the worm is of some importance; the larger the worm, the quicker it reacts. In this work, worms of about the same size were therefore selected. These and many other conditions which could not be analyzed must surely affect the reaction.

Number of Reactions. Even when used as a direct comparison between solutions, the factor of fatigue enters. In order to determine for how many trials a single worm could be used, eight worms were subjected to forty trials each. At the end of that time they were beginning to show fatigue. The reaction-time was lengthening. They seemed to be stimulated but pulled themselves out of the solutions with difficulty. Hence, worms should not be used for many reactions; better not above twenty-four, for after this the reaction-time lengthens.

Stimulation by Distilled Water. Although worms can live in water for days, they withdraw rapidly from it if possible (see Table II). So the fact that they can maintain life does not mean that

water is not stimulating, as Towle ('04) and others have said. The following work was suggested by that of G. Bullot ('04). He speaks of the toxicity of pure distilled water and says this is ordinarily due to the dissolved copper salts. He determined, however, that very pure water which he distilled several times, using Jena glass, quartz, and platinum stills, continued to have a toxic effect on Gammarus. The distilled water supply available for my work was kept in copper vats. Some distilled water was redistilled carefully, using Jena glass stills, one containing potassium permanganate and the other sulphuric acid. The water was collected through block tin condensers, received in clean Jena flasks and used the same day. It was compared with ordinary tap water and ordinary distilled water (see Table I). The tap water was less stimulating than the distilled water and slightly more stimulating than the redistilled water.

TABLE I

AVERAGE REACTION-TIME IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO TAP, DISTILLED, AND REDISTILLED WATER. DEC. 17

Number of the worm	Water		
	Distilled	Tap	Redistilled
I	9.1	8.4	12.7
II	8.7	12.0	13.2
III	11.6	14.3	15.1
IV	12.8	16.6	15.0
Average	10.5	12.8	14.0

Physiological Solution. Not only is distilled water more stimulating than tap water, but the latter is more effective than dilute hydroxide solutions (see Table II, page 388).

Parker and Metcalf ('06) found that for salts dilute solutions were less stimulating than water. An attempt was made to find a solution which was physiological for worms. Parker and Metcalf ('06, p. 67) have shown that worms which live in manure and in

TABLE II

AVERAGE REACTION-TIME IN SECONDS FOR EIGHT TRIALS EACH OF A SERIES OF EARTHWORMS TO A SOLUTION OF POTASSIUM HYDROXIDE AND WATER. FEB. 26

Number of the worm	H_2O (Before KOH)	KOH $\frac{m}{250}$	KOH $\frac{m}{350}$	KOH $\frac{m}{400}$	H_2O (After KOH)
I	12.2	5.5	12.0	19.8	12.4
II	11.7	3.9	24.4	51.5	13.2
III	10.8	7.2	27.0	15.6	20.0
IV	14.4	6.0	14.0	22.8	10.6
Average	12.3	5.6	19.3	27.4	14.0

garden soil differ, a fact which they attribute to the chemical environment. If this were so, a solution of the inorganic salts in approximately the same concentration as in manure should not be stimulating.

Professor Parker kindly obtained for me through the New Haven Agricultural Department the following analysis of manure (Wolff):

K	.53	NO_3	.58	H_2O	71.3%
Na	.10	Cl	.04	Organic	25.4%
Ca	.21	SO_4	.07	Other	3.3%
Mg	.14	PO_4	.28		
$Fe(OH)_3$	.11	Silica	1.27		
Al_2O_3					
	1.09		2.24		100.0%

On this basis, molal solutions of the nitrates of potassium, sodium, calcium, and magnesium were made. The solution employed contained the following amount of salts:

KNO_3	N	98.59 c.c.
$NaNO_3$	N	31.89 c.c.
$Ca(NO_3)_2$	2N	38.89 c.c.
$Mg(NO_3)_2$	2N	42.73 c.c.
H_2O		787.90 c.c.
		1000.00 c.c.

This solution is practically decinormal as regards the potassium, but almost three times as concentrated (.294N) in respect to the nitrates. This was further diluted and tried in different strengths. The longest reaction-time, over three minutes in some cases and averaging one and a half minutes, occurred in 1/50 concentration of the solution. In both greater and less concentrated solutions the reaction-time was shorter. In the later experiments ten cubic centimeters of this solution S/50 was kept in the jars; this did not affect the reactions of the worms to the hydroxide, as was found by controls in water, but did give more even results and seemed to keep the worms in better condition. These solutions are obviously only a rough approximation to a physiological solution. In S/50 the concentration is:

$$K = \frac{m}{500}, Na = \frac{m}{1500}, \quad Ca = \frac{m}{1250}, \quad Mg = \frac{m}{1250}, NO_3 = \frac{m}{175}.$$

No attempt was made in this work to vary the concentration of the kations nor to introduce other anions. This could be done on theoretical consideration and would probably give better results. However, this solution is interesting in that it is different from the Ringer solutions commonly used. The behavior to it seems to bear out the evidence just quoted, that reaction to neutral salts is dependent on the chemical environment. This solution is certainly very different from the concentration of salts in sea water.

Temperature. It is a striking fact that the literature makes so little reference to the effect of temperature on worms. Towle ('04) mentions casually that cold retards the action. It is well known that the speed of chemical reactions is increased with the rise of temperature. This has been worked out in minute detail for the effect on the periods of latency, contraction and relaxation of both smooth and striated muscle. It has been shown that

temperature affects both the irritability and velocity of conduction of nerves. In general, the rule of Van't Hoff may be applied; i.e., that for every ten degrees rise in temperature, the velocity of reaction increases from one to two fold for physical processes and from two to three fold for chemical reactions.

Experiments in which the temperatures were varied indicated a marked effect which was constant and important. The results are shown in Table III and plotted in Figure 1.

TABLE III

AVERAGE REACTION-TIME IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO SODIUM HYDROXIDE $\frac{m}{500}$ AT VARYING TEMPERATURES

Number of the worm	17° C.	21° C.	24° C.	27.5° C.
I	30.2	42.0	22.5	19.2
II	23.9	8.9	12.0	9.7
III	16.7	10.6	13.7	6.6
IV	25.1	20.8	12.9	7.8
Average	23.9	20.6	15.3	10.8

Small variations in the temperature made a marked difference in the reaction-time, which shortens as the temperature increases; i.e., the higher the temperature, the more the stimulation. With a rise of ten degrees, the velocity of the reaction is a little more than doubled. This would place the response in the category of chemical reactions.

In the later work a thermostat was employed. A large basin of water was heated with an electric light. The temperature was regulated to within half a degree by a mercury bulb which dipped into the water and made and broke the current in the light. The water was kept at a uniform temperature throughout by a glass suction stirrer run by a small motor. The whole was contained in a double-walled, padded chamber. This kept the air at about the same temperature as the water. The apparatus was placed in the

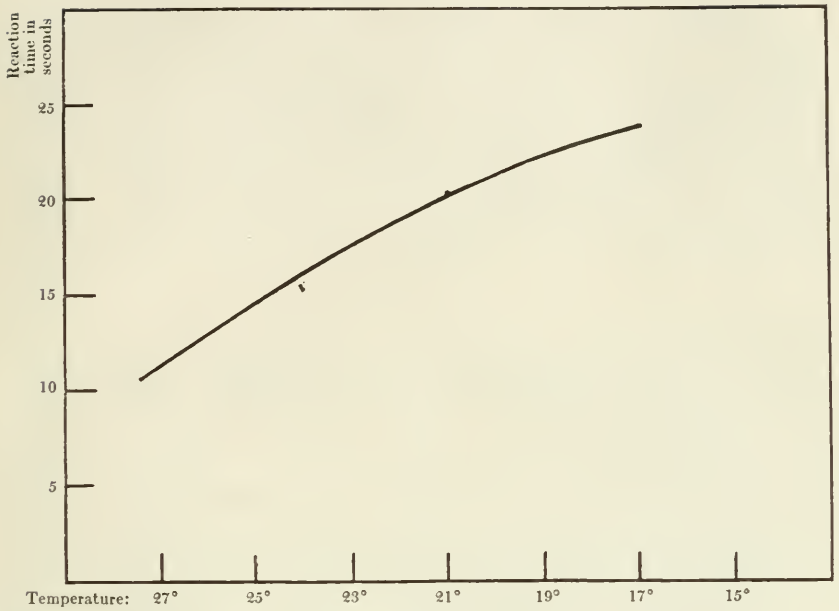

FIGURE 1. Effect of Temperature (plotted from Table VII). Reaction time in seconds.

water so that the solutions were kept at constant temperature. All subsequent work was done at 25 degrees C.

IV. Effect of Hydroxides on Reaction-time

The object of this part of the work was to determine the effect of varying concentrations of sodium and potassium hydroxides on the earthworms and then to compare their effects at the same dilution. First, semi-normal sodium hydroxide was tried. The first worms showed signs of intense irritation; they threw out yellow, malodorous secretions all over their bodies and died immediately. They withdrew from a twentieth normal solution the way a person would from fire. The limits of the reaction lie approximately between $\frac{m}{200}$ and $\frac{m}{800}$. At the latter dilution they remained in the solution a considerable time; below the former dilutions they withdrew so rapidly that no accurate reaction-time could be taken.

The effect of NaOH is shown in Table IV, graphically plotted in Figure 2. The results show that with varying concentration of sodium hydroxide employed, the stimulus depends directly on the amount of the base present. The effect of KOH is shown in Table V, and in Figure 3. The results with potassium hydroxide are practically identical with those found for sodium hydroxide. These reactions were repeated a number of times and the cases selected are typical ones. So the conclusion can be drawn that for these

TABLE IV

Average Reaction-times in Seconds of a Series of Earthworms, for Eight Trials each, to Varying Concentrations of Sodium Hydroxide. Feb. 18, 25° C.

Number of the worm	$\frac{m}{350}$	$\frac{m}{400}$	$\frac{m}{450}$	$\frac{m}{500}$
I	6.5	22.5	22.4	27.2
II	2.0	7.4	27.5	34.9
III	1.0	10.0	16.3	13.3
IV	0.8	13.5	16.1	20.0
Average	2.5	13.3	20.5	23.8

TABLE V

Average Reaction-time in Seconds of a Series of Earthworms, for Eight Trials each, to Varying Concentrations of Potassium Hydroxide. Feb. 21, 25° C.

Number of the worm	$\frac{m}{350}$	$\frac{m}{400}$	$\frac{m}{450}$	$\frac{m}{500}$
I	7.9	5.8	12.2	10.9
II	—	20.0	23.0	29.8
III	21.5	27.0	24.0	19.0
IV	14.0	27.0	28.0	38.0
Average	14.4	19.9	21.8	24.4

dilutions the speed of the reactions is directly proportional to the concentration of the base, — the stronger the solution, the quicker the worm withdraws.

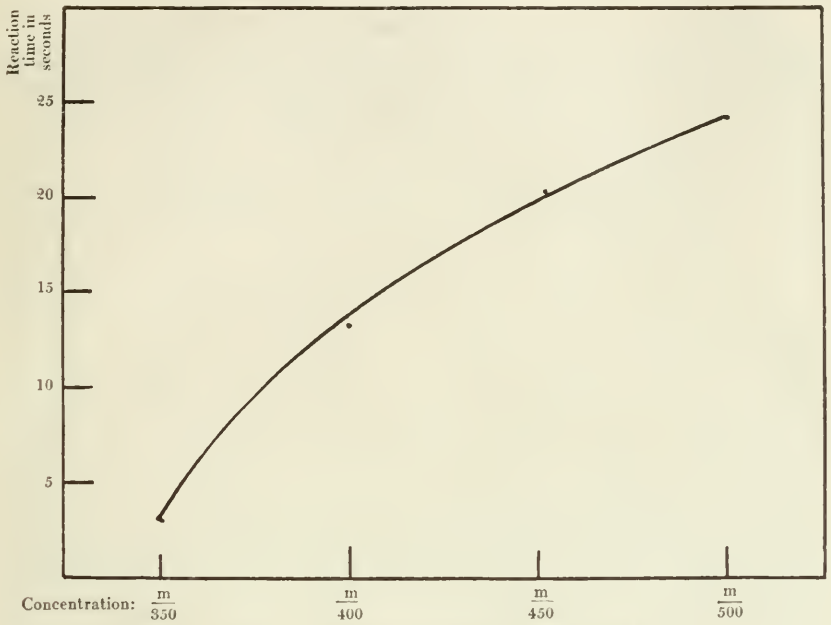

FIGURE 2. Effect of Sodium Hydroxide (plotted from Table IV). Reaction time in seconds.

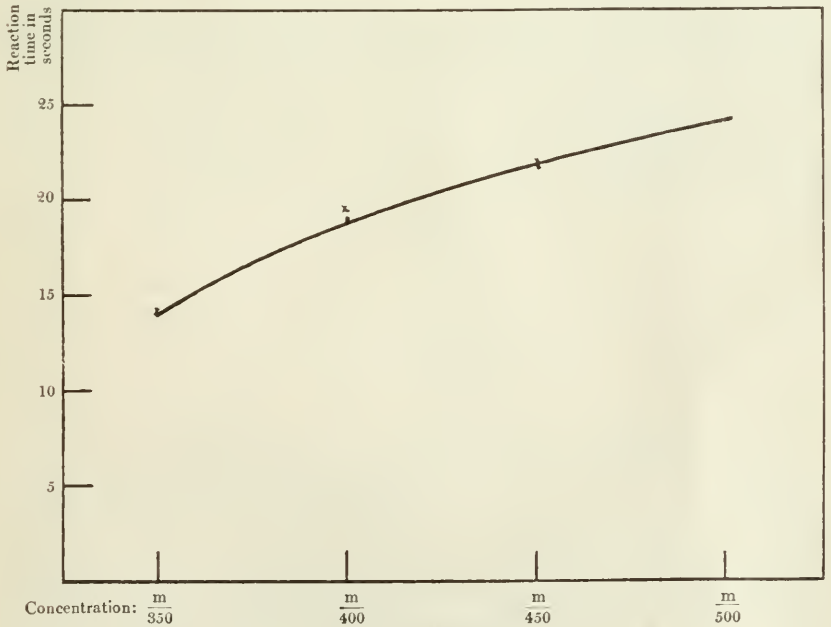

FIGURE 3. Effect of Potassium Hydroxide (plotted from Table V). Reaction time in seconds.

That this is not true for all concentrations has already been pointed out when it was shown that the worms withdrew more quickly from water than from dilute solutions. Beyond a dilution greater than $\frac{m}{1000}$ the reaction-times shorten till the effect of pure water is met. There is a point of dilution at which the hydroxide which is present retards the reaction-time as compared with pure water. This means that there is more than one factor involved.

Since it was known that sodium and potassium hydroxide (within the limits of the dilutions used) were both stimulating in proportion to the amount of base present, it was necessary to have a direct comparison between the two solutions of the same strength to determine their relative effects. The procedure was as in previous cases, except that the different basic solutions were used instead of varying concentrations of the same base. The two solutions were not of quite the same strength, due to differences in the stock solutions, but this difficulty was overcome by interpolating the true value from the curve obtained from plotting the values of different concentrations. (This is justifiable from the uniformity of results.)

The hydroxides of sodium and potassium in the same concentrations are compared in Tables VI and VII. This shows that the reactions are very nearly equal.

The reaction-time is somewhat shorter with sodium than with potassium hydroxide.

$$\frac{NaOH\ \frac{m}{400}}{KOH\ \frac{m}{400}} = 93\%$$

$$\frac{NaOH\ \frac{m}{300}}{KOH\ \frac{m}{300}} = 99\%$$

At $\frac{m}{300}$ the KOH is more dissociated than NaOH and hence should be more stimulating (see page 28) if the stimulation is due only to the hydroxyl ions, but as Parker and Metcalf ('06, p. 58) showed that sodium ions are more stimulating than potassium ions, it may be deduced that the effect was due mainly to the

TABLE VI

Reaction-times in Seconds of a Series of Earthworms, for Eight Trials each, to Sodium Hydroxide $\frac{m}{400}$ and Potassium Hydroxide $\frac{m}{400}$. May 14 and 16. 25° C.

Number of the worm	NaOH $\frac{m}{400}$	KOH $\frac{m}{450}$	Correction Factor KOH $= \frac{m^1}{400}$
I	25.9	29.1	
II	20.9	29.4	
III	32.2	40.5	
IV	35.3	43.5	
Average	28.5	35.6	30.0

[1] The correction factor is obtained by calculating the relative value for the desired concentration from the curve plotted in Fig. 3.

TABLE VII

Reaction-times in Seconds of the Series of Earthworms, for Eight Trials each, to Sodium Hydroxide $\frac{m}{300}$ and Potassium Hydroxide $\frac{m}{300}$. May 27 and June 7. 25° C.

Number of the worm	NaOH $\frac{m}{300}$	KOH $\frac{m}{340}$	Correction Factor KOH $= \frac{m^1}{300}$
I	38.8	55.3	
II	19.0	20.3	
III	27.0	48.1	
IV	43.0	45.5	
Average	31.9	42.3	32.15

[1] The correction factor is obtained by calculating the relative value for the desired concentration from the curve plotted in Fig. 3.

hydroxyl ions. However, since the sodium is slightly more stimulating than the potassium hydroxide, the effect is an additive one in which both kations and anions take part.

V. New Method

The desire to test more clearly the relative effects of the katıons, as well as to get a more constant series of readings, led to the devising of a new method, which I have called the fence method. Many other schemes were tried, but the plan finally adapted was to divide a rectangular glass tray (such as is used in photography) into two compartments by a paraffine partition a quarter of an inch wide. A notch about three-quarters of an inch long and reaching to within half an inch of the bottom was cut out of this partition. Such a notched fence may obviously vary in three ways, — first in height, second in length, and third in thickness. Care must be taken that the notch is not cut too deep, or there will not be enough liquid on either side to cover the worms. If it is not deep enough they cannot reach the bottom easily. It was found that the best lining for the bottom was a paraffine coating. By this procedure the solutions on the two sides of the fence do not mix; they do not even wet the walls.

The worms were not threaded as in the former method, but were simply picked up with a moist toothpick. They were so placed that the clitellum rested on the partition fence, the anterior end in one solution and the posterior in the other. Care must be taken to have the source of light at right angles to the long axis of the dish, for the worms are negatively phototropic.

Under such circumstances a worm can do one of several things: it can stay where it was put; it can enter a solution; it can withdraw from it. If it does the last, it can use a swinging motion like that of an elephant's trunk, or a muscular retraction like a collapsing accordion. It may make all of these movements. Time was recorded as in the previous work. In this series of reactions, in all cases, the head was placed in the hydroxide solution.

This method necessitated but little handling of the worms and therefore they were in a more normal state. Their response is a more natural one, for instead of lifting themselves out of a solution, they crawl out. Loeb ('05, pp. 453 and 479) admitted that his method of determining toxicity was faulty, because it depended on a negative factor, — the cessation of irritability. This proce-

dure gives positive results. Further, it gives a direct comparison between any two solutions.

Fresh solutions were made up before trying this method, so as to avoid the use of the correction factor.

Methyl-orange:	Phenolphthalein:
KOH 1.04 $\frac{m}{2}$	1.01 $\frac{m}{2}$
NaOH 1.04 $\frac{m}{2}$	.957 $\frac{m}{2}$

The reactions with this method agree well. Table VIII shows a few records taken at random to show the actual results of individual reactions.

TABLE VIII

Individual Reaction-times in Seconds of Earthworms to NaOH $\frac{m}{300}$.

Number of trial	Worm I	Worm II
	March 19 19° C.	March 23 15° C.
I	10.0	8.0
II	13.0	8.0
III	12.4	8.0
IV	8.0	8.0
V	5.0	11.0
VI	15.0	6.2
VII	14.0	7.8
Average	11.0	8.0

The Effect of Hydroxides. The work on the comparison of the hydroxides was repeated with this method. The results are more nearly equal than those obtained in the former method. The sodium hydroxide was $\frac{m}{310}$ instead of $\frac{m}{300}$. Hence, this would tend to obscure the difference slightly since the sodium hydroxide, being very slightly weaker, would be less stimulating.

TABLE IX

AVERAGE REACTION-TIMES IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, FOR WITHDRAWING FROM NaOH $\frac{m}{310}$ AND KOH $\frac{m}{300}$ INTO WATER. MAY 3. AND MAY 21. 18° C.

Number of the worm	NaOH $\frac{m}{310}$ | H_2O	KOH $\frac{m}{300}$ | H_2O	NaOH $\frac{m}{310}$ | H_2O
	Before		After
I	6.9	6.4	6.5
II	3.0	2.6	3.0
III	7.7	8.0	8.0
Average	8.0	7.5	8.0

There were no data for making a correction factor as in the former work, for no comparative results on varying concentrations of the same solution were undertaken. The solutions were so nearly equal that this inequality falls within the experimental error. The results are given in Table IX. They show that the reaction-times are very nearly equal, — slightly longer for sodium than potassium (see page 28).

$$\frac{\text{NaOH } \frac{m}{310}}{\text{KOH } \frac{m}{300}} = 106\%$$

Relative Stimulation of Kation and Anion. By the former method it was shown that the effect of the solutions was in the main due to the OH ions and that the Na ions and K ions had a slight but characteristic effect. Loeb ('05, pp. 463 and 476) is of opinion that it makes no difference to what basic ions the hydroxyl ions are attached, since they are to be considered the stimulating factor. The results with all hydroxides are the same and the hydroxyl is poisonous in dilutions when the basic ion is non-toxic. To test this experimentally, a solution of c.p. NaCl $\frac{m}{300}$ was used,

against a solution of NaOH $\frac{m}{300.}$. If the solutions depended for their stimulating property on the sodium, they would be equal. In every case the worms withdrew into the solution containing the NaCl; not only that, but faster than into water alone. In other words, the salt solution was less stimulating than water. Hence, the stimulation is due principally to the hydroxyl ion.

VI. Consideration

The experimental evidence in this investigation points to the fact that NaOH and KOH in dilute solutions are stimulating mainly through the hydroxyl ion and are about equally stimulating for Allolobophora foetida. It remains to consider what can be deduced from this and whether the theories suggested by other investigators can be applied to explain the phenomena.

The reaction is more complicated than it seems at first. It is probably mainly the result of direct stimulation of the sensory mechanism in the epithelium. This leads to a motor reflex causing the animal to withdraw from the stimulating solution. There may be some effect due to the fact that the skin acts as an imperfect semi-permeable membrane.

The hydroxides used were sodium and potassium. These bases when in solution dissociate into Na + and K + basic ions, and OH — hydroxyl ions. The degree of ionization is nearly the same for each. The ionization increases with the dilution and is measured by the specific conductivity. This increase is slightly greater for sodium than for potassium, though the latter is actually more dissociated. The percentage dissociated at $\frac{m}{200}$ is 91.5 per cent for NaOH and 95.0 per cent for KOH; at $\frac{m}{500}$ it is 93.0 per cent for NaOH and 95.8 per cent for KOH. Increase in temperature also increases ionization. At this dilution it is only a fraction of a per cent per degree, but slightly larger for NaOH than for KOH. Hence the conditions of the experiment make the ionization very nearly equal. The difference is slightly in favor of greater ionization for KOH, so that in equal concentrations there would be a

slightly larger number of OH ions in the potassium hydroxide than in the sodium hydroxide solution.

The action of the hydroxyl ion must next be considered. The fact that OH is a strong protoplasmic stimulant has been shown by Loeb ('05, p. 480). It can be tasted in solutions of .006 gr. ions per liter (Höber, '11). Kahlenberg ('98) says OH is sapid at $\frac{m}{400}$ while NO_3 must be as concentrated as $\frac{m}{6}$ to be tasted. Alkalies increase the absorption of water under all conditions (Loeb, '05, p. 516). It also acts as a strong lytic for protoplasm (Lillie, '06). The following is a classification of anions from several widely divergent points of view:

Swelling	Br, Cl, CO_3, NO_3, CH_3COO, SO_4, $C_4H_4O_6$, PO_4 (Fischer)
Swelling	SO_4, Cl, NO_3, Br, I, CNS, BrO_3, OH (Lillie)
Antiprecipitating effect	SO_4, PO_4, Cl, NO_3, Br, I, CNS (Pauli)
Taste	SO_4, NO_3, Br, I, BrO_3, OH (Kahlenberg)

In all these cases the order is almost identical for swelling, antiprecipitating and tasting. The OH group is the most effective.

There have been many theories as to the way in which these solutions stimulate. Braeuning ('04) said that stimulation in the frog depends on the diffusion coefficient. KOH $\frac{m}{200}$ = 1.68 and NaOH $\frac{m}{200}$ = 1.12. Our reactions were so similar that this cannot be important.

Loeb ('05, p. 475) maintained that the toxicity is dependent on the migration velocity. K = 64, Na = 23, OH = 173. Hence, $\frac{NaOH}{KOH} = \frac{196}{237}$. This is not borne out by the facts.

The osmotic pressure has often been declared to be the cause of stimulation. Loeb's ('05, p. 470) work showed that muscles swelled in hypotonic solutions and shrank in hypertonic ones for the first hour. This increases more rapidly than it ought in hypotonic solutions and decreases more slowly than it ought in hypertonic ones. However, isosmotic chlorides of Li, K, Rb, Cs, Mg, Ca, Sr and Ba all have the same effect, so that osmotic pressure must have some effect. Overton showed that plasmolysis in plants did not follow osmotic pressure, and Greeley ('04) showed

that ionic effects prevail over the osmotic effects. Loeb ('05, p. 511) also showed that under certain conditions the osmotic law was not true, for he found that even in a 2.8 per cent salt solution, at the end of eighteen hours, a muscle which had decreased in weight at first, afterward weighed more than normal. He showed further that there was a specific ionic effect KCl 0.7 per cent, causing a 45 per cent increase in eighteen hours, and CaCl 0.7 per cent a 20 per cent decrease for the same time, — whereas in LiCl 0.7 per cent the muscle remained constant. This is also evident from a consideration of the reactions obtained by Parker and Metcalf ('06, p. 58) and those in this paper. If the reaction were dependent on osmotic pressure, regardless of the actual length of time, the *longest* reaction in each solution would be in the same concentration. For KCl this occurs at $\frac{m}{50}$; for KOH at $\frac{m}{800}$.

Animal tissue is not an ideal semipermeable membrane. There must be some diffusion. Loeb ('08) has said that in hypertonic solutions, water goes one way and *ions* the other. This would imply, if the action were selective, a condition similar to that of the red blood corpuscles. Lillie ('10, '11) has shown that stimulation causes a change in the permeability of the membranes, so that pigment will diffuse into the solution. This he has called the "sensitizing effect." This runs in the same order as other protoplasmic effects (see page 29), except that he says OH, H and some other non-ionic compounds form irreversible reactions, whereas the anion effects are reversible. If this sensitizing effect were so, the reactions would show a shortening. In fact, we have often noticed that the first reaction is followed by shorter ones for the first few reactions. Then the reactions become constant. Hence the OH cannot destroy the cell membrane in the solutions used. Fischer ('10) has doubted whether there is any membrane, permeable or semipermeable, around the cell. He says it is merely a matter of ions on colloids as influenced by absorption.

Pauli ('07) has shown beautifully the effect of ions on the coagulations of protein, and on the electric charge. Anions are anti-coagulators and electronegative. They act as antitoxins to the kations. Greeley ('04), Lillie ('11a, '11b), Mathews ('04a, '04b, '05), and Sutherland ('06) have applied these principles to

biological problems with excellent results. They conclude that stimulation is dependent on the action current produced by electric charges of the sum of the acting ions. The present work is in agreement with such conclusions.

In this paper an attempt has been made to analyze only one of the factors involved; that is, the cause of the specific effect of the electrolytes as a stimulant. At least two other aspects of the problem present themselves: first, why should certain solutions be non-stimulating? and, second, why should water be more stimulating than dilute solutions containing irritating ions? As far as the electrolytes are concerned there seems to be no doubt that in solutions of hydroxides the effect of the basic ion is of relatively little importance and the solutions are stimulating approximately in proportion to their hydroxyl ion concentration.

VII. Summary

1. Individual dung worms vary in their reaction-time.

2. The average reaction-time varies on different days.

3. Distilled water is more stimulating than redistilled water or tap water.

4. Water is more stimulating than dilute solutions.

5. Worms can be well handled in a "physiological dung solution."

6. Temperature is an important factor; the higher the temperature the faster the reaction.

7. The "fence method" gave more accurate results than the suspension method.

8. Hydroxides are stimulating in proportion to their hydroxyl ions.

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CARBON DIOXIDE PRODUCTION FROM THE NERVE FIBER IN A HYDROGEN ATMOSPHERE

By SHIRO TASHIRO and H. S. ADAMS

[*From the Laboratory of Bio-chemistry and Pharmacology, The University of Chicago, and the Marine Biological Laboratory, Woods Hole, Mass.*]

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Introduction

AMONG certain facts which are difficult to reconcile with the physical theory of nerve conduction, the relation of oxygen to the state of excitability is the most important one. In spite of many contrary findings, the facts indicating a dependence of irritability on oxygen in general are now accumulating. Critical studies made by Verworn and his school not only support the idea that irritability is primarily a chemical phenomenon, but also clearly point out some of the fundamental errors which were involved in the earlier work, and were responsible for the present discrepancy.[1]

Under the direction of Professor A. P. Mathews, Tashiro has demonstrated not only that all living nerves give off CO_2, but also that their metabolism increases on stimulation.[2] To demonstrate a further relation between the state of irritability and the metabolic activity of the nerve, we have already reported the facts that nerves stimulated by very weak concentrations of an anaesthetic produce more CO_2 than normally, but when anaesthetized by a higher concentration, which produces a reversible loss of irritability, their CO_2 production is greatly depressed.[3] Whether the different rate of the nerve metabolism is the cause or effect of the change in the state of excitability, it is certain that an

[1] Verworn: Irritability, Yale University Press, 1913, pp. 102, 182.

[2] Tashiro: This journal, 1913, xxxii, p. 107.

[3] *Ibid.*, 1914 xxxiii, p. xxxviii.

active metabolism in the nerve fiber is a fact and the incorrect assumption of an absence of metabolism can no longer be used by the physical school to support their argument.

It is known from the investigations of Verworn and his colleagues that the irritability of nerves is dependent on oxygen; since it has been shown by Tashiro that a very close relation exists between irritability and the power of increasing CO_2 on stimulation, it was very important to determine whether lack of oxygen, which thus lowers irritability, would inhibit or diminish the CO_2 production on stimulation, and whether the CO_2 of resting nerves will be diminished in a hydrogen atmosphere.

Methods and Material

Hydrogen was used in place of air, no particular reason being attached to its use in preference to nitrogen except that of convenience. The gas, furnished by Eimer and Amend, was prepared by the electrolysis of distilled water. It was washed through permanganate, distilled water, and alkaline pyrogallate, and collected by displacement in a large carboy, over a 20 per cent caustic solution, so that every trace of CO_2 might be removed. The gas was further tested for freedom from CO_2 in each experiment with the biometer. This carboy of hydrogen was used in place of CO_2 — free air, and with it the apparatus was filled in exactly the same manner as described elsewhere.[1]

The claw nerve of the Spider Crab was selected on account of our fairly extensive knowledge of its metabolic rate. The nerve is isolated in the manner already mentioned, quickly weighed, and allowed to respire in the apparatus, usually for ten minutes in this gas, and its CO_2 production is quantitatively estimated. The apparatus used exclusively in these experiments was the biometer (apparatus II), with which amounts of CO_2 as small as .000,0001 gm. can be measured.

CO_2 Production from the Resting Nerve in Hydrogen

It appears from this table that in a medium deficient in oxygen the claw nerve of Spider Crab gives off less CO_2 than in the

[1] Tashiro: *loc. cit.*, p. 139.

TABLE I

CO_2 Production from Resting Claw Nerve of Spider Crab, Libinia Canaliculata, in Hydrogen

Date	Temp. of room	Wt. of nerve	Duration of respiration	Vol. of gas taken from respiratory chamber	Ppt. of $BaCO_3$ after 10 mins.	No. of c.c. which gives no ppt., calculated for 10 mg., 10 mins.	No. of c.c. which gives ppt., calculated for 10 mg., 10 mins.	Original vol. of respiratory chamber
Sept. 2	23.8°	37.0 mg.	10 mins.	1.0 c.c.	–	3.70		15. c.c.
"	23.1°	40.5 "	"	1.0 "	–	4.05		"
"	22.8°	27.0 "	"	2.0 "	+		5.40	"
Sept. 3	23.4°	70.0 "	"	.9 "	+		6.30	"
"	23.5°	31.5 "	"	1.0 "	+		6.30	"
"	23.9°	45.5 "	"	1.2 "	+		5.46	"
"	24.1°	33.5 "	"	1.8 "	–	6.03 [1]		"
Sept. 4	22.8°	49.5 "	"	1.0 "	+		4.95	"
"	23.0°	32.0 "	"	1.5 "	+		4.80	"
"	23.0°	36.0 "	"	1.2 "	+		4.32	"
"	22.0°	34.0 "	"	1.2 "	–	4.08		"
"	21.4°	42.0 "	"	1.1 "	+		4.62	"

maximum error

1.0×10^{-7} gms. $\times$ 15/4.32 = 3.4×10^{-7} gms. ($- 0.2 \times 10^{-7}$ gms.)

at 23°.

In air: 6.7×10^{-7} gms. (15–16°)

7.9×10^{-7} gms. (20.2°)[2]

[1] This nerve has been kept in hydrogen for ten minutes, previous to the experiment.

[2] Taken from results obtained in connection with "Effect of Anaesthetics," now in press.

atmosphere. This may mean one of three things, — CO_2 production is diminished, (1) because of lack of oxygen, or (2) on account of the effect of hydrogen, or by 1 and 2 combined. This important point could be decided by substituting in place of hydrogen other inert gases, such as nitrogen. It is to be regretted that we could not perform these experiments because of the lack of facilities at Woods Hole. A variety of facts, however, lead to the inference that it is not due to the effect of the hydrogen. The inertness of hydrogen, which the physiologist experiences in the case of the nerve and other tissues in general, strongly supports our contention that the lowering of the CO_2 output in hydrogen is not due to the presence of hydrogen, but results from the lack of oxygen.

Just what the CO_2 production in hydrogen represents is a matter for further experiment. Two factors may be responsible; either diffusion of already formed CO_2 fixed in the nerve, or its production as a regular end product of metabolism going on anaerobically. In the case of the muscle, where CO_2 is produced continuously in hydrogen, and often is increased temporarily at the outset, the gas is believed to be formed in part by the decomposition of bicarbonate in the tissue, on account of the formation of lactic acid which begins as soon as oxygen is taken away.

That the CO_2 in our experiment must be the expression of a certain phase of metabolic activity which continues to exist without oxygen even in aerobic tissue is not a matter of reckless speculation. The possible mechanism of metabolic activity in the nerve fiber has been considered by Tashiro[1] somewhat as Vernon has suggested in the case of other tissues. He has shown by the action of drugs the possibility of two more or less independent phases of metabolism, one the formation of an organic peroxide between the tissue and oxygen, and the other the decomposition of this peroxide by peroxidase.

From results recently obtained on stimulation of the respiratory center by "oxygen want," Gasser and Loevenhart came to a somewhat similar conclusion.[2] They divide the oxidative process

[1] *Loc. cit.*, p. 135.

[2] Gasser and Lovenhart: Journal of Pharmacology and Experimental Therapeutics, 1914, v, p. 272.

into two phases; one an "activity process" having to do with functional activity, which does not require free oxygen, the other a "recovery process" which consists of the storing up of potential energy and the removal of products of activity, which requires fixation of oxygen by the cell.

Accordingly we may look upon the CO_2 production in hydrogen as a sort of expression of half of the normal metabolism. This mechanism probably will persist long after functional activity has ceased. For not only in hydrogen, but also in conditions of narcosis we have shown that the nerve nevertheless continues to give off CO_2 although in greatly diminished amounts during loss of excitability.[1]

CO_2 Production from "Stimulated" Nerves in Hydrogen

In addition to many physiological facts brought forth by Verworn and his school, Haberlandt has observed that the sciatic nerve of the Frog, at 14° to 19° C. takes up oxygen, — 33.4 to 41.7 c.cm. per gm. per hour. Furthermore, when this nerve is excited the intake of oxygen is increased.[2] Buijtendijk found a similar fact for the cranial nerve of certain fishes, in which the oxygen intake is increased by electrical stimulation.[3] That oxygen is involved in such stimulation of some nerves, at least, seems to be fairly definitely established. Mathews found that salts either would not stimulate a nerve, or their power of stimulation was greatly reduced, if the nerve remained in the body for a time after death, or if the nerve were brought into the salt solution in an atmosphere of hydrogen.

By the preceding it has been shown that CO_2 production is reduced in the resting nerve in hydrogen, and this reduction,

[1] How long such a process can go on without oxygen must depend on the rate of metabolism and the presence of some mechanism to remove the toxic by-products of such metabolism. We show elsewhere that tissues which can stay active in an oxygen-free atmosphere for any length of time have a comparatively low rate of metabolism, even in the air. See "CO_2 production in ganglionated cord of limulus heart," now in press.

[2] Haberlandt: Archive für Physiologie, 1911, p. 419.

[3] Buijtendijk: Koninklijk Akademie van Wetenschappen, Amsterdam, afd. xix, pp. 615–621.

TABLE II

CO_2 Production of Claw Nerve of Spider Crab, Libinia Canaliculata, when Stimulated in Hydrogen

Date	Temperature of room	Wt. of nerve in mgs.	Duration of respiration	Stimulation	Vol. of gas taken from respiratory chamber	Ppt. of $BaCO_3$ after 10 mins.	No. of c.c. which gives no ppt., calculated for 10 mg., 10 mins.	No. of c.c. which gives ppt., calculated for 10 mg., 10 mins.	Original vol. of respiratory chamber
Sept. 3	24.0°	39.5	10 mins.	Stimulated 10 mins.	1.2 c.c.	+		4.74	15 c.c.
"	23.8°	32.0	"	Stimulated first 5 mins.	1.2 "	–	3.84		"
"	22.7°	36.0	"	Stimulated last 5 mins.	1.3 "	–	4.68		"
"	21.6°	35.0	"	Stimulated 10 mins.	1.2 "	+		4.20	"

1.0×10^{-7} gms. $\times$ 15/4.2 = 3.6×10^{-7} gms. (at 21.6°).

judging from the evidence cited, ought to be accompanied by a diminution of irritability. The next question to be attacked was whether there would be an increase of CO_2 output by a nerve electrically stimulated in hydrogen. For this purpose also the claw nerve of the Spider Crab was chosen. Stimulation was effected by an induction coil in a manner similar to that described in earlier papers, using approximately the same strength of current as in air.

TABLE III

COMPARATIVE RATES OF CO_2 PRODUCTION IN THE NERVE WITH AND WITHOUT OXYGEN

Nerve	Medium	CO_2 from resting nerve, calculated for 10 mg.— 10 mins.	CO_2 from stimulated nerve, calculated for 10 mg.— 10 mins.
Claw nerve of spider crab	CO_2 free air	6.7×10^{-7} gms. (15–16°)	16×10^{-7} gms. (14–16°)
" " " " "	" " "	7.9×10^{-7} gms. (20.2°)	
" " " " "	CO_2, free Hydrogen	3.4×10^{-7} gms. (23.0°)	3.6×10^{-7} gms. (21.0°)

The results given in Table II bring to light an interesting fact. It appears that there takes place practically no increase of CO_2 when the nerve is "stimulated" in hydrogen with weak induction shocks. A close inspection of the table shows possibly a very slight increase of CO_2, which may be due perhaps to experimental error, or is surely within the limits of error. It is certain that there is no such enormous increase as is observed in the case of the nerve stimulated in air.

These results suggest two possible interpretations, — either the nerve when in hydrogen was not stimulated by the same strength of current as stimulated the same nerve in the air, or the CO_2 production from the resting nerve in hydrogen is so small that stimulation under these conditions involves an increase so slight as not to be detected, and the nerve by continued stimulation is quickly exhausted.

Fröhlich[1] found that when a sciatic nerve of a frog is deprived of atmospheric oxygen its irritability, measured by the threshold of stimulation for muscle contraction, decreases more and more, until after the lapse of some hours the stimulation required is so strong as to approach the region of the "stromschleifengreze." If such is the case, the claw nerve also to which is applied such a weak current may be not in reality stimulated. On the other hand Thörner,[2] taking the action current as an index, found that a nerve continuously stimulated in an atmosphere deficient in oxygen is quickly exhausted. It is remarkable that the action current in nitrogen falls to two-thirds of its original value within the first ten minutes. Fatigue of the nerve by continuous stimulation during the first few minutes of our experiments with hydrogen may be brought about.[3]

Whatever interpretation we take, and as a matter of fact both factors may doubtless enter in here, the fact that there is no decided increase of CO_2 on a weak electrical stimulation in hydrogen points inevitably to the view that oxygen is a primary factor in the excitability of the nerve, as well as in the conductance of the nerve impulse.

Discussion

The evidence set forth here that in the absence of oxygen the claw nerve of the Spider Crab has a far lower CO_2 output than in air, and that the application of a weak current, which stimulated the nerve in air and more than doubled its CO_2 output, fails under these conditions to give any decided increase is further proof for the argument that the primary basis of protoplasmic irritability must be a chemical one, as is contended by Mathews, Verworn and others. So far there exist no decided evidence against this metabolic basis of irritability, except the lack of heat production in the case of stimulated nerves so carefully studied by A. V. Hill.[4] This fact, however, we shall later have opportunity to con-

[1] Quoted from Verworn's Irritability, p. 102; see Zeitschrift f. allgem. Physiologie, 1904, Bd. III.

[2] *Ibid.*, p. 185; see *Ibid.*, 1908, Bd. VIII.

[3] Unfortunately we could not test this point on account of lack of time and accommodation in Woods Hole.

[4] A. V. Hill: Journal of physiology, 1912, xliii, p. 433.

sider. The fact remains that there is perfect parallellism between the state of excitability and the CO_2 output, to which the following experimental facts, brought out by studies on the CO_2 production of nerves under various conditions, bear evidence:

1. All irritable tissues give off CO_2, resting nerves being no exception.

2. When irritable tissue is stimulated, this CO_2 production is increased.

3. When a nerve is treated with weak concentrations of anaesthetics, which are known to stimulate or increase irritability, the CO_2 production is likewise increased.

4. When the nerve is treated with higher concentrations, in which reversible loss of irritability takes place, CO_2 production is diminished.

5. In an oxygen-free medium the claw nerve of the Spider Crab shows an exceedingly low CO_2 output.[1]

6. "Stimulation" by a weak electric current in any oxygen-free medium fails to produce any marked increase in CO_2.

Our results on CO_2 production in hydrogen depend on experiments with the Spider Crab nerve only, and are not of themselves sufficient to permit generalization, but they are at least sufficient to indicate that oxygen is primarily concerned with metabolism in the nerve, and they are in harmony with the view that the real basis of irritability may be a chemical one.

We take great pleasure in acknowledging here our indebtedness to Professor A. P. Mathews, under whose direction a series of investigations regarding gaseous exchange in nerve systems has been undertaken, of which series this paper is one.

[1] Some physiologists have called to my attention the possibility that CO_2 production in an isolated new fiber is not a physiological expression, but rather is due to tissue destruction accompanying death; and the increase of CO_2 on stimulation may be due to an acceleration of the death process. The decrease of CO_2 production in oxygen free medium, together with perfect parallelism between CO_2 production and general state of excitability of normal nerve in situ under the similar conditions, should be sufficient evidences against such an argument. For the general consideration on the nature of survival respiration of a tissue, we refer to FLETCHER works: Journal of physiology, 1898–9, xxiii, p. 10.

THE CONDITIONS DETERMINING THE RATE OF CONDUCTION IN IRRITABLE TISSUES AND ESPECIALLY IN NERVE

By RALPH S. LILLIE

[*From the Laboratory of General Physiology, Clark University*]

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THE state of excitation travels in the form of a wave along the irritable element at a rate which is well known to vary greatly from tissue to tissue and from organism to organism. We find, however, when we examine any particular conducting tissue, that the rate of conduction resembles the other physiological properties of the tissue in having a specific character of its own, and that when temperature and other external conditions are constant its value does not vary widely from a certain definite mean. This rate shows no appreciable correlation with ionic or other diffusion-velocities, nor with the transmission-velocities of mechanical, thermal, or electrical influences. It is, however, greatly influenced by changes of temperature, the temperature-coefficient being approximately that of chemical reaction-velocities, and also by changes in the composition of the medium — being retarded, for instance, by anaesthetics. It appears, therefore, that some chemical process or processes must play an important part in the transmission of excitation from one region of an irritable element to another. Any general theory of the nature of the physiological conduction-process must take into account *all* of the above peculiarities and cannot be based on conditions observed in a single irritable tissue alone. A broadly comparative consideration is most likely to lead to theoretical conceptions that will be valid in all cases. Hitherto the nature and conditions of physiological conduction have been investigated chiefly in nerve; but the problem is a general one and in no sense peculiar to this tissue.

It is clear that in any irritable element that responds as a whole to a local stimulus the process initiated at the point of stimulus must spread over the entire element. The manner in which this spread takes place and the conditions determining its velocity in any special instance are the subjects of discussion in the present paper.

The comparative observations on the rate of conduction are most complete for nerve. There are also numerous measurements for striated and heart muscle and fewer for smooth muscle and other tissues. In nerve the velocity of the impulse is greatest in warm-blooded vertebrates, where it may exceed a hundred meters per second, and slowest in sessile animals like mollusca, where it is often no more than a few centimeters per second. Certain general physiological correlations are highly significant. We find throughout the animal kingdom a direct relation between the quickness of muscular response and the velocity of propagation of impulses along the conducting elements.[1] The biological advantage of this is perhaps not so obvious as would appear at first sight; it would seem that what is needed for quickness of motor response is quickness of muscular contraction rather than quickness of nerve conduction, provided this is adequate; yet it is apparently a general rule that where muscular contraction is rapid nerve conduction is also rapid and *vice versa; i.e.*, in any animal rapidity of response is a characteristic of the whole neuromuscular apparatus and not only of certain separate elements. This general fact indicates that there is some condition common to both conducting and contractile elements which determines at the same time both quickness of response and quickness of conduction.

Rapid response implies brevity of latent period and rapid rise of the excitation-state to its maximum. In nerve the only tangible index of the local nerve-process is the electrical variation; the rate at which this variation rises at any point of the nerve from zero to its maximum is a measure of the velocity of the local nervous disturbance. Now the rate of rise of the action-current shows a close parallelism with the velocity of conduction, as the examples about to be cited will illustrate. That the bioelectric process should show this relation to a process of apparently quite different

[1] Carlson: American Journal of Physiology, 1904, x, p. 401; 1906, xv, p. 136.

nature, the propagation of the excitation-wave, suggests the existence of a direct interdependence, *i.e.*, that the electrical variation as such is the essential change on which the conduction depends. This view was in fact favored by Du Bois-Reymond, Hermann, Kühne, and other early students of the bioelectric phenomena.[2]

There is, however, the possible alternative that some other underlying process determines both the electrical variation and the transmission of the impulse; the electrical variation would on this view be merely an index of the underlying physiological change and might be of no special importance in itself. Such a view appears more consistent with the obvious fact that the propagation of the excitation-wave is readily blocked by conditions that have no influence on electrical conduction, such as ligaturing or locally narcotizing the nerve. On the other hand, the transmission of the state of excitation from one irritable tissue to another by means of the action-current of the first is an equally familiar phenomenon, occurring with perfect constancy under appropriate conditions, and affording direct proof that this kind of transmission is at least possible. Whether or not it is the normal method of transmission of excitation from point to point along the nerve or muscle-fibre is the question which I propose to discuss in the present paper. The existence of blocking effects of the kind just cited is not necessarily inconsistent with this hypothesis, as I shall attempt to show later;[3] it is quite possible, for instance, that electrical effects at the site of the block may interfere with or compensate those that form the basis of the transmission. I shall first review the facts that indicate a dependence of transmission on the bioelectric variations of the active elements, and shall afterwards consider the possible manner in which this transmission may take place.

First, with regard to the parallelism between the velocity of the electrical variation and the velocity of conduction in various irritable tissues. The following table summarizes a large number of observations made by different investigators under different condi-

[2] Du Bois-Reymond: Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysik, Vol. ii, p. 698; *cf.* p. 733. Hermann: *cf.* Handbuch, Vol. 1, p. 256; Vol. 2, i, p. 193. Kühne: *cf.* Croonian Lecture: Proceedings of the Royal Society, 1888, Vol. xliv, p. 446; Zeitschrift für Biologie, xxiv, p. 383.

[3] See page 440.

TABLE

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
A. Striated Muscle:		
Frog's gastrocnemius ...	2.58–3.84 σ [4] (*ca.* 20°)	
" " ...	1.1–3.5 σ [5] (*ca.* 20°)	*ca.* 3–4 met-.sec.[5]
" sartorius	1.6–3.2 σ [6] (*ca.* 20°)	
" "	4.1–4.2 σ (8°)[7]	*ca.* 1.2 met.-sec. (8°)[7]
" "	2.4–2.9 σ (18°)[7]	*ca.* 1.65 met.-sec. (18°)[7]
" "	13 σ (3°)[8]	1.06 met.-sec. (3°)[8]
" "	5.8 σ (14.8°)[8]	1.65 met.-sec. (14.8°)[8]
" hyoglossus	20 σ (3°)[8]	0.38 met.-sec. (3°)[8]
" "	8.9 σ (14.7°)[8]	*ca.* 0.96 met.-sec. (14.7°)[8]
Mammalian muscle		
Rabbit's gastrocnemius ..	*ca.* 2 σ (*ca.* 37°)[9]	10–13 met.-sec. in man's forearm [10]

tions. The second column gives the time occupied by the rise of the action-current curve from zero to its apex, as measured usually with the thread galvanometer. The third column gives the velocity of propagation of the excitation-wave. The table includes not only observations on various normal tissues at different temperatures, but also a number of observations on narcotized

[4] SNYDER: American Journal of Physiology, 1913, xxxii, p. 336.

[5] HERMANN: Archiv für die gesammte Physiologie, 1877, xv, p. 233; propagation-rate in frog's gastrocnemius determined by Matthias: *ibid.*, 1892, liii, p. 70; his experiments give a range of 3.27 to 6.36 met.-sec.

[6] GARTEN: Winterstein's Handbuch der vergleichenden Physiologie, Bd. III, 1910, p. 113.

[7] K. LUCAS: Journal of Physiology, 1909, xxxix, p. 207. The propagation-velocities are not given by Lucas, but are estimated from his curves.

[8] BABKIN: Archiv für die gesammte Physiologie, 1908, cxxv, p. 595.

[9] GARTEN: *loc. cit.*, p. 114; Zeitschrift für Biologie, 1909, lii, p. 534.

[10] HERMANN: Archiv für die gesammte Physiologie, 1876, xvi, p. 410; MATTHIAS: *ibid.*, 1893, liii, p. 70.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
B. Nerve:		
Frog's sciatic	0.9–1.2 σ (*ca.* 18°)[11]	20–40 met.-sec. at 20° [12]
" "	0.55 σ (32°)[11]	30–80 met.-sec. at 30° [12]
Rabbit's sciatic	*ca.* 0.5 σ (32°)[13]	*ca.* 100 met.-sec. at 37°
Dog's "	*ca.* 0.7 σ (36°)[13]	*ca.* 100 met.-sec. at 37°
Non-medullated (splenic of horse).....	*ca.* 60–70 σ [14]	*ca.* 0.47–0.54 met.-sec.[14]
Olfactory of pike	*ca.* 70 σ (12°)[15]	60–90 mm.-sec. (5°) 118–150 mm.-sec. (13°) 160–240 mm.-sec. (20°)[16]
Commissural of anodonta.	*ca.* 200 σ [17]	*ca.* 2.5 cm.-sec. (varying estimates from 1 to 5 cm.-sec.)[17]
Mantle-nerve of octopus.	8.2–11.3 σ [18]	2.5–3.5 met.-sec. in O. vulgaris [20]
" "	*ca.* 20 σ [19]	*ca.* 2 met.-sec. in O. punctatus [21]

and fatigued tissues. It will be seen that in all cases the two values show a closely parallel variation in different animals, and also in the same animal under varying external conditions. Tissues whose normal bioelectric variations are slow exhibit slow conduc-

[11] GARTEN: Winterstein's Handbuch, *loc. cit.*, p. 137.

[12] SNYDER: American Journal of Physiology, 1908, xxii, p. 179.

[13] GARTEN: *loc. cit.*, p. 139.

[14] GARTEN: *loc. cit.*, pp. 144–6.

[15] GARTEN: *loc. cit.*, p. 141.

[16] NICOLAI: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 65; Archiv für Anatomie und Physiologie, Physiol. Abth., 1905, Suppl., p. 341.

[17] GARTEN: *loc. cit.*, p. 142.

[18] FUCHS: Sitzungsberichte der kais. Akad. d. Wiss. Wien, 1894, Bd. 103, Abth. 3, p. 207.

[19] BORUTTAU: Archiv f. die gesammte Physiologie, 1905, cvii, p. 193.

[20] GOTCH: Schäfer's Text Book, Vol. II, p. 482; FUCHS: *loc. cit.*

[21] JENKINS and CARLSON: American Journal of Physiology, 1903, viii, p. 262.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
C. Cardiac Muscle:		
Ventricular muscle of mammal	10–15 σ [22] (body temperature)	Averages apparently 2–4 met.-sec.[23]
Ventricular of frog	40–60 σ [24] (*ca.* 18°)	From 50–200 mm.-sec.[25]
D. Smooth Muscle:		
Retractor penis of dog	*ca.* 2 sec.[26]	1–7 mm. sec.; average *ca.* 5 mm.-sec.[26]
Ureter-muscle	0.2–0.4 sec.[27]	Average *ca.* 14–15 mm.-sec.[27]

tion-rates and *vice versa;* and whatever condition retards the velocity of the local bioelectric process also retards the velocity of propagation.

These data appear to be sufficiently extensive and varied to leave no doubt that a close positive correlation exists between the rate at which the electrical variation rises to its maximum at any region of the excited tissue, and the rate at which the excitation-wave is propagated in that tissue. It is not immediately evident why this should be the case. Conceivably the physiological change

[22] The duration of the upstroke of the prominence in the electrocardiogram called R in EINTHOVEN's terminology. *Cf.* EINTHOVEN: Archiv f. d. gesammte Physiologie, 1908, cxxii, p. 517; KAHN: *ibid.*, 1909, cxxvi, p. 197, cxxix, p. 291; SAMOJLOFF: Archiv für Anatomie u. Physiologie, Physiol. Abth., 1910, pp. 508–9.

[23] BAYLISS and STARLING, SCHLÜTER, WALLER and REID: For references *cf.* NAGEL's Handbuch der Physiologie, i, p. 250.

[24] Estimated from the duration of the upstroke of the ventricular variation R in SAMOJLOFF's electrocardiograms. *Cf.* Archiv für Physiologie, 1910, p. 507; Archiv f. d. gesammte Physiologie, 1910, vol. cxxxv, p. 417; *ibid.*, 1912, cxlvii, p. 249.

[25] ENGELMANN, BURDON-SANDERSON. *Cf.* NAGEL'S Handbuch, i, p. 250. The velocity varies of course with temperature.

[26] VON BRÜCKE: Archiv f. d. ges. Physiol., 1910, cxxxiii, p. 313.

[27] ORBELI and VON BRÜCKE: *ibid.*, p. 341.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
E. Influence of Narcotics: [28]		
Frog's sciatic	Normal: 3.2–4 σ (8.9°); partly narcotized: 3.4–4.4 σ [29]. Normal *ca.* 4 σ; narcotized 5.2–6σ [30]	Average 17.3 met.-sec. in normal; *ca.* 12 met.-sec. in weakly, 9.4 met.-sec. in more strongly narcotized nerves.[29]
Pike's olfactory.....	Normal 55–60 σ (*ca.* 9°)[29] partly narcotized: 67–82 σ	Average normal velocity: *ca.* 81 mm.-sec.; in narcosis *ca.* 59 mm.-sec.[29]

determining propagation might be quite independent of the bioelectric process,[31] just as, for example, it appears in nerve to be independent of heat-production. The above evidence, however, plainly points to the existence of a close connection between the two. Garten, Fröhlich, and Lucas have pointed out this parallel-

[28] Fatigue also decreases both propagation-velocity and rate of electrical variation in muscle. *Cf.* von Brücke: Archiv f. d. ges. Physiol., 1908, cxxiv, p. 215. This is probably the case whenever an impulse is conducted with a decrement.[30] Fuchs also notes the lengthening of the action-current curve in the fatigued nerves of cephalopods (*loc. cit.*).

[29] Koike: Zeitschrift für Biologie, 1911, lv, p. 311. It will be noted that according to Koike's curves the duration of the rising phase in the frog's nerve is only slightly decreased during narcosis; the slope of the curve, however, and hence its total height, are much lower (two-thirds or less) than in the normal nerve; and the retardation of propagation-velocity is probably due chiefly to the decrease in the amplitude of the variation.

[30] Fröhlich: Zeitschrift f. allg. Physiologie, 1912, v, xiv, p. 55. Boruttau and Fröhlich (Archiv f. d. ges. Physiol., 1904, xv, p. 444) also show that both the rate of rise and the amplitude of the electrical variation are decreased during narcosis.

[31] Ellison has in fact recently maintained that the electrical variation can be abolished in nerve by cinchonamine hydrochloride without interfering with conduction (Journal of Physiology, 1911, xliii, p. 28); but his work has not received confirmation. *Cf.* Dittler and Satake: Archiv f. d. ges. Physiol., 1912, cxliv, p. 229; also Lucas' critique, Proc. Roy. Soc., B. 1912, lxxxv, p. 503.

ism,[32] but without attempting an analysis of its exact significance. Such an analysis is evidently necessary to any complete consideration of the nature of the processes of stimulation and conduction, and I shall accordingly attempt it in what follows.

It is possible to explain this correlation in two essentially different ways. First, it may be supposed that the rate of the rise and subsidence of the electrical variation at any point of the conducting tissue is simply an index of the time which the excitation-wave takes to pass that point. This view regards the electrical variation as a mere accompaniment or sign of an underlying process which is transmitted in the form of a wave along the tissue. Obviously the more rapidly this wave passes a given point the more rapidly the associated bioelectric process will rise to its maximum and subside at that point. On the other hand, it is possible that the electrical variation is itself the essential feature of the excitation-process, and constitutes that functional component of the local process which directly excites the adjoining regions of the tissue to activity; if so, its rate of development must determine the rate at which excitation is transmitted from the active region of the tissue to those adjoining. This possibility has been hitherto largely disregarded; it is, however, consistent with all that we know of the conditions of stimulation, and recently the evidence in its favor has greatly increased. As I shall attempt to show below, this view accounts satisfactorily both for the transmission of the excitation-wave, and for the wide variation in the rate of this transmission in different tissues.

It should be pointed out that according to this view not only the rate of development or time of rise of the local electrical variation but also its total amplitude (or voltage) is a factor in determining the rate of conduction. This amplitude is, however, the less variable of the two factors; and hence the correlation between the propagation-velocity of the excitation-wave and the rate of development of the action-current is much more clearly evident than that between propagation-velocity and amplitude. But in some cases, as in Koike's observations with narcotized nerves cited above, the chief factor in reducing the propagation-

[32] GARTEN: Winterstein's Handbuch, *loc. cit.;* FRÖHLICH: *loc. cit.;* LUCAS: *loc. cit.*

velocity appears to be the decreased range of the variation rather than the decreased duration of its rise. This period is not greatly shortened in the experiments of Koike, while the height of the action-current curve is reduced to two-thirds or less of its normal value. In the majority of instances, however, the rate at which the variation rises to its maximum is apparently the essential or preponderant factor in determining the rate of conduction.

As already said, this correlation is readily intelligible if the transmission of the excitation-wave is a direct consequence of the electrical variation as such. If we suppose that for transmission of excitation from the already active region of the nerve to an inactive region 2 centimeters (*e.g.*) distant the existence of a certain minimal potential-difference between active and inactive regions is required, *e.g.* 20 millivolts, then the more rapidly this potential-difference is attained the more rapid will be the transmission. Now in point of fact a current between platinum electrodes 2 centimeters apart differing in potential by 20 millivolts or even less is amply sufficient to excite a sensitive nerve. If we assume that the voltage of the action current is 30 millivolts, then the current flowing along the nerve between these two electrodes is approximately equal to that flowing between a region, A, already active (and therefore negative) and an inactive region, B, 3 centimeters distant. If this action-current, at the instant when it reaches its maximum, causes excitation at this distant point B, and the resulting negative variation there reaches its maximum in .001 second, a second excitation-impulse will be transmitted from B to a point 3 centimeters further along the nerve in the same time, .001 second. Three centimeters in .001 second is in fact the approximate velocity of the nerve impulse in frog's nerve at 20°. It thus appears possible that the velocity of propagation is a function (1) of the rapidity with which the excited region undergoes its electrical variation, — *i.e.*, assumes externally a certain negative potential relatively to the unexcited regions, and (2) of the maximal distance along which the current passing between this temporarily negative region and the as yet unexcited (positive) regions of the nerve can make itself felt as a stimulus. This distance will depend on a number of variable conditions, — the amplitude of the electrical variation, the electrical resistance of

the tissue, its threshold of stimulation, the rate of rise of the stimulating current to its full intensity, the electrical condition of the nerve in the intermediate region. According to this hypothesis, in the time t (in seconds) after stimulation there would be electrical stimulation of the nerve at a point B, s centimeters distant from the already active region A; point B would then become after the same interval the source of stimulation for a third point, C, s centimeters beyond, and this process would continue. The velocity of propagation in centimeters per second would then be measured by the quotient s/t, where t is the time between stimulation and the attainment of a certain critical point in the action-current curve, and s the maximal distance at which the current between active and as yet inactive regions just suffices to stimulate.

It will be noted that this view takes no account of the differences in the intensity of the electrical stimulus imparted by the action-current to the nerve at different distances from the active region. There is, however, strong evidence that the intensity of the electrical stimulus is a matter of indifference in the stimulation of a normal nerve, provided the stimulus reaches the threshold value: *i.e.*, that the "all-or-none" law applies to the normal nerve fibre.[33] Hence any stimulus that excites at all will call forth a response of full intensity. The *maximal* distance from the active region at which the branch of the action-current causes excitation is thus the only one to be considered. For transmission of the above conceived kind it is in fact essential that the "all-or-none" law should apply at all points of the nerve. Under other conditions the impulse would inevitably die out,[34] as in fact appears to be the case in a narcotized stretch of nerve. It appears highly probable that the "all-or-none" form of local response is indispensable to any tissue whose activity depends on conduction of the excitation-state to some distance from the point of stimulation.

[33] *Cf.* Gotch: Journal of Physiology, 1912, xxviii, p. 395; Veszi: Zeitschrift f. allgemeine Physiologie, 1912, xiii, p. 321; Adrian: Journal of Physiology, 1913, xlv, p. 321; Lodholz: Zeitschrift für allgemeine Physiologie, 1913, xv, p. 269.

[34] Compare my discussion of the mechanism of transmission in my former paper on the present subject: American Journal of Physiology, 1911, vol. xxviii, pp. 217 *seq.*

On the present view, therefore, normal physiological stimulation is identical with electrical stimulation, and the spread of the excitation-state depends directly upon the bioelectric variation accompanying the local stimulation, the velocity of this spread being a function (1) of the sensitivity and electrical conductivity of the tissue and (2) of the amplitude and time-relations of the bioelectric variation. Numerous biological facts are in harmony with this hypothesis besides those already cited, and I shall now proceed to review briefly what seem to be the most significant of these.

It has been known since the work of Matteucci and Du Bois-Reymond that the electrical variation accompanying the activity of one irritable tissue can stimulate another tissue. An active frog's muscle can thus stimulate another muscle or a nerve; the action-current of the ventricular beat can stimulate a sensitive nerve muscle preparation, and so on. There are limits to the possibilities of this kind of excitation; ordinarily an active nerve does not stimulate a muscle, and the action-current of smooth muscle will not stimulate a nerve. Apparently there is needed a certain correspondence between the time-relations of the electrical variation in the stimulating tissue, and the time-factor of electrical excitation in the responding tissue. The rise of the electrical variation in the one tissue may be too gradual to stimulate another tissue which requires a rapid rate of change in the stimulating current; or its duration may be too brief, even though its rate of development and intensity may be sufficient. Thus the electrical variation of the frog's ventricle may stimulate a nerve [35] (especially one cooled so as to prolong its time-factor of excitation), but that of the nerve is too brief to stimulate this kind of muscle. There is thus here a certain relation of irreciprocality in the transmission,

[35] KÜHNE found the action-current of the turtle's heart (*Testudo græca*) incapable of stimulating a very sensitive frog's nerve, though able to excite the curarized frog's sartorius. On the other hand, the action-current of the rabbit's heart proved very effective as a stimulus to frog's nerve. Kühne rightly ascribes the difference to the difference in the time-relations ("zeitliche Verlauf") of the action-currents of the two hearts. Similarly with the failure of the action-current of smooth muscle to stimulate nerve. *Cf.* KÜHNE: Untersuchungen aus dem physiologischen Institut der Universität Heidelberg, 1879, Bd. iii, pp. 55, 87 *seq.*

— which suggests a possible basis for the irreciprocality of conduction between motor and sensory nerve-cells in the reflex arc.[36]

Under appropriate conditions the transmission of the excitation-state from one irritable element to another by means of the former's action-current takes place with perfect constancy. Now conduction means simply the excitation of one region of a continuous element by some process occurring in the adjoining active region; and we must therefore examine more closely the possibility that the electrical variation is the essential change that causes this excitation. The action-current is the only known change accompanying excitation in nerve which is competent to stimulate an adjoining stretch of nerve, and it is known that under certain conditions one nerve may in fact be stimulated by the action-current of another.[37] There is also good evidence that the character and intensity of the local excitation-process undergo no essential change as it passes along nerve or muscle fibres. At the point of external stimulation the process has the same characteristics as in regions that enter into activity by "conduction" from adjoining excited regions.[38] It is clear that the local excitation-process includes some functional component which acts as stimulus on adjoining regions and produces there the same effect as does the external stimulus. That this component is the electrical variation is indicated not only by the above general considerations but by various facts of comparative physiology to be partly cited below.

Before citing these facts it seems desirable to consider the

[36] The action current of a rapidly responding tissue (or cell) may not last long enough to stimulate a slowly responding tissue; while that of a slowly responding tissue is always sufficient, as regards mere duration, to stimulate the rapidly responding; the slowly rising is less effective than the rapidly rising action-current only if its rate of rise is so gradual that the requisite current-strength is not attained within the characteristic time-period of the responding tissue. Similar considerations of course apply to the interactions between adjacent cells. See below, p. 439.

[37] Hering: Sitzungsberichte d. Akad. Wiss. Wien, math-naturw. Kl., 1882, Vol. lxxxv, 3te Abth. p. 237; v. Uexküll: Zeitschrift für Biologie, 1894, xxx, p. 184 *ff.* *Cf.* also Biedermann: Electrophysiology, English translation, Vol. 2, pp. 264 *seq.*

[38] *Cf.* Lucas: Journal of Physiology, 1906, xxxiv, p. 51; Bramwell and Lucas: *ibid.*, 1911, xlii, p. 495. The propagated disturbance is associated with a refractory period similar in all respects to that at the site of stimulation.

alternative hypothesis that some chemical or "molecular" change forms the direct basis of transmission. This hypothesis appears to be a favorite with many physiologists, but it has the disadvantages both of being vague in itself and of having no satisfactory analogues in inorganic processes. The rapid transmission of chemical effects, as in an explosion, is seen on closer analysis to be a secondary consequence of the transmission of local changes of temperature or pressure, or both combined. In a train of gunpowder, for instance, the rate of transmission depends mainly on the rate of conduction of heat between the ignited and the adjoining unignited areas, combustion beginning when the temperature reaches the ignition-point. In other explosions additional factors enter: *e.g.*, the mechanical shock resulting from the local reaction, and the development of pressures which are transmitted with high velocity; such transmission may be much more rapid than that depending on local rise of temperature alone.[39] It is obvious that in such a tissue as nerve, where mechanical and thermal changes are absent or insignificant, these factors cannot enter. The only other known local process that can serve as a basis for the transmission is the electrical one. It is significant that in the cases of "chemical action at a distance" described by Ostwald [40] the basis of transmission is electrical; this is also the case in the spread of the catalytic action over the surface of the mercury in the rhythmical hydrogen peroxide catalysis of Bredig; according to Antropoff,[41] this transmission is due to electrolytic action at the boundary of the surface-film of mercury peroxidate; this action automatically dissolves the film at that region, and the process thus initiated then spreads over the entire surface. I have already pointed out some of the many remarkable analogies between this process and

[39] The high velocity of the explosion-wave in guncotton, gases confined in tubes (where it may exceed 2000 meters per second), etc., is due to the development and rapid transmission of high pressures, with accompanying generation of heat. *Cf.* Will: Zeitschrift für Elektrochemie, 1906, xii, p. 558. Also Nernst's Theoretical Chemistry and Mellor's Chemical Statics and Dynamics for a more general account of explosive action.

[40] Ostwald: "Chemische Fernwirkung": Zeitschrift für physikalische Chemie, 1891, ix, p. 540.

[41] Antropoff: Zeitschrift für physikalische Chemie, 1907, lxii, p. 513.

the processes of physiological excitation and conduction.[42] The progressive dissolution of the film of peroxidate covering the mercury is a good instance of that kind of chemical action which depends on conditions essentially characteristic of surfaces. The potential-difference between the film-covered mercury-surface and the adjoining peroxide solution differs from that between the free metallic surface and the solution by *ca.* 0.12 volts; hence when the free mercury surface is exposed — *e.g.*, by local mechanical rupture of the film — a current flows between these two regions, which exerts electrolytic action and reduces the film to the condition of metallic mercury. Such an effect necessarily spreads, at a rate depending on the velocity of the chemical or electrolytic process by which the film is locally removed (or sufficiently altered). Similarly the potential-difference between the living cell and its surrounding medium depends on the characteristics of the protoplasmic surface-film; and when this is altered locally — *e.g.*, when its permeability is increased — the potential-difference at that point is also altered and a current flows between the altered and the unaltered regions. This current, on the present hypothesis, is the condition of propagation of the surface-change that determines excitation. The processes on which the propagation depends are thus closely similar as regards determining conditions in the two cases, although the comparison must of course not be pushed too far into detail.[43]

In addition to the experimental facts already cited, there is much general biological evidence that the transmission of excitation in many other living tissues and cells is due to the electrical variations of the active elements. One muscle may be stimulated by the action-current of another; for such transmission contact or close proximity of the tissues is necessary, but not direct continuity of irritable elements.[44] Transmission of excitation from

[42] *Cf.* American Journal of Physiology: 1909, xxiv, p. 18.

[43] Du Bois-Reymond concluded — from the fact that electrical stimulation is a polar phenomenon — that the primary effect in stimulation is an electrolysis. *Cf.*: Untersuchungen über thierische Electricität, vol. ii, p. 387: "Galvanische Reizung ist uns nichts mehr als die erste Stufe der Elektrolyse eines Nerven." Compare the suggestion made below, p. 444.

[44] *Cf.* Kühne: Croonian Lecture, *loc. cit.* p. 446: "Two (curarized) muscles . . . need only be pressed together transversely over a narrow area to make a

cell to cell without any organic continuity is in fact a widespread phenomenon, and one difficult to explain except as the result of electrical excitation by the action-current. There is the case of transmission from neurone to neurone across the synapses; but disagreement still exists as to whether the interlacing dendrites are continuous or not, so that this instance might be regarded as equivocal. The propagation of waves of increased activity over the surface of a ciliated epithelium is perhaps a better instance; increased ciliary activity in one area excites adjoining areas to increased activity, so that a certain synchrony tends to be preserved between neighboring cells. If ciliary activity, like other forms of contractility, is due to variations of electrical polarization at the surfaces of the contractile elements,[45] an action-current must accompany each ciliary stroke, and its stimulating influence will be transmitted through the medium for some distance. Some observations which I made at Naples a number of years ago,[46] on the swimming-plates of the ctenophore *Eucharis*, support this view: the successive plates of a single row beat in order, so that waves of movement continually run along the row from the aboral to the oral end; if a strip is cut from the body of the animal, containing a row of plates together with a portion of the underlying jelly, and the piece of tissue is left for some hours in sea-water, the mass of jelly contracts and rounds off, and frequently in such a way that the opposite ends of the row are brought within a few millimeters of each other. The rounded portion of jelly is then encircled by a row of still active plates. When this condition is reached it frequently happens that the wave of ciliary movement is transmitted across the interval between the opposite ends of the row, so that the movement continues to travel round and round the ring of tissue in the same direction, often for hours at a time.

single muscle of them of double length in which the stimulation and contraction are propagated from one end to the other. Since the transference from one muscle to the other is done away with as soon as we bring the finest gutta percha between the muscles as an insulator, or gold leaf as a secondary circuit, the first muscle must have excited the second electrically."

[45] *Cf.* my paper on the conditions of activity of the ctenophore swimming plate, in American Journal of Physiology, 1908, xxi, p. 214 *seq.*

[46] Briefly reported in the Year-Book of the Carnegie Institution, No. 4, 1905, p. 282.

The interval across which the stimulus may thus be transmitted may be half a centimeter or more. The time is too short for regeneration of a special conducting tissue (supposing this to exist); the exciting influence is evidently transmitted through the sea-water and jelly; and nothing but an electrical influence is known to act in this manner. Another striking instance of transmission of excitation without organic continuity between the active elements is furnished by the spermatozoa of certain marine animals, especially of the annelid *Nereis*. The spermatozoa of *Nereis*, if collected in watch-glasses, gather together into numerous small groups or aggregations shortly after shedding from the body of the animal. After standing for a few minutes such an aggregation settles down into a layer, and it is then found that the separate spermatozoa forming this layer exhibit a marked tendency to beat in unison with one another; a regular and synchronous beating thus becomes established, the whole group of cells resembling in this respect a ciliated epithelium; and waves of accelerated movement are transmitted from cell to cell just as in such an epithelium.[47] It is difficult to conceive of any influences other than the electrical which can cause these cells thus to transmit their rate of movement to one another; if, however, each stroke of a flagellum is accompanied by an action-current whose influence extends for a short distance through the surrounding sea-water and is sufficient to stimulate neighboring spermatozoa, the phenomenon becomes at once intelligible.

The facts just cited afford evidence that the excitation can be transmitted from one irritable element to another through the surrounding medium independently of any organic connection between the elements. The media of irritable tissues, however, characteristically contain salts, and the withdrawal of these is well known to arrest activity in many cases, especially in muscle

[47] F. R. Lillie: Journal of Experimental Zoölogy, 1913, xiv, p. 523. There is also evidence that adjacent blastomeres undergoing mitosis influence one another, so that a certain synchrony tends to be maintained, which disappears when the cells are separated. *Cf.* M. Sorokina: "Uber Synchronismus der Zelltheilungen," Archiv f. Entwicklungsmechanik, 1912, xxxv, p. 30. If cell-division is accompanied by changes in the electrical polarization of the cell-surface, such transmission is readily explained in the present hypothesis.

and nerve, — tissues in which it is essential that the local disturbance should be propagated for some distance if the irritable element is to react as a whole. Brünings has attributed part of the action of sugar solutions in temporarily depriving muscle and nerve of their normal properties to a lowering of the electrical conductivity of the media;[48] if the propagation of the excitation-impulse depends on this condition, it is easy to see why irritability is temporarily lost in solutions of non-conductors. Under normal conditions the electrical variation which accompanies activity may be conducted for a considerable distance through the medium with an intensity sufficient for stimulation. The precise degree to which this is possible depends on the electrical conductivity of the medium as well as on several other factors, chief of which are the sensitivity of the irritable elements to electrical stimulation, and the intensity and time-relations of the electrical variation. When electrical conductivity is lowered to a sufficient degree, transmission of excitation is no longer possible.

The idea that organic continuity is necessary for the transmission of excitation is derived more especially from the conditions in nerve, where the conducting elements are typically continuous, and where severing this continuity prevents the passage of the impulse. But that transmission is possible without this continuity is sufficiently clear from the facts already cited. There are, however, advantages in continuity of the kind shown by nerve-fibres if the conduction is to be *rapid*. The presence of cross-partitions at intervals would decrease the electrical conductivity of the tissue in the longitudinal direction and thus lessen the distance along which the local electrical effect could be transmitted with an intensity sufficient for excitation. According to Hermann the electrical resistance of frog's nerve in the direction *across* the fibres (*i.e.*, with partitions — or surface-films — interposed) is five times as great as in the lengthwise direction.[49] There is in fact

[48] *Cf.* Brünings: Archiv f. d. gesammte Physiologie, 1907, cxvii, p. 418.

[49] Hermann: Archiv f. d. gesammte Physiologie, 1872, v, p. 223. The same is true of muscle. In dead muscle and nerve this difference disappears or is greatly diminished. Hermann refers the resistance to the presence of polarizable elements or membranes in the tissues; this polarizability largely disappears on the death of the tissue. Polarizability, as we now know, is a function of semi-permeability, which is lost at death.

a marked decrease in the velocity of conduction of the nerve-impulse between different neurones, a condition quite possibly due in part to the interposition of the relatively impermeable and hence non-conducting surface-films of the adjoining synapses.

We have now to consider the question: are the characteristics of the local electrical variation (*e.g.*, in nerve) such that excitation through its means of an inactive region of nerve situated at some distance from the active region (of at least 2 or 3 centimeters) is possible? In order that an electric current traversing an irritable tissue may cause excitation it must have a certain minimal strength, a certain minimal duration of flow, and a certain minimal rate of change of intensity. We have therefore to inquire whether that portion of the action-current which traverses the nerve at the above distance from the immediate site of activity has these characteristics.

First, with regard to the intensity of the current flowing between excited and unexcited regions. The usual law relating current-strength to voltage and resistance will apply. The intensity of the current between excited and inactive regions will thus decrease as the distance between the two regions increases, being at any point inversely proportional to the electrical resistance between that point and the excited region.[50] The current will thus be strong near the site of activity, decreasing as the distance from this increases; there will thus be a distance beyond which it will be ineffective as a stimulus. The question to be answered is: what is this maximal distance? The problem can only be approached indirectly. The following considerations seem to point the way to an approximate solution. The electromotive force of the action-current of nerve is approximately equal to that of the demarcation-current (*ca.* 0.03 volt in frog's nerve). Now the length of the stretch of nerve by traversing which the demarcation-current can actually cause excitation may be very considerable, in favorable cases 2 centimeters or more.[51] This is shown by the well-

[50] This resistance will in a cylindrical nerve be directly proportional to the distance.

[51] HERING obtained twitches with a distance of 25 millimeters between the clay pads across which the nerve lay. The nerve current was closed by means of a vessel containing salt solution brought up from below. *Cf.* HERING: Sit-

known experiment where the resistance of the external portion of the demarcation-current circuit is suddenly decreased or increased by connecting cross-section and longitudinal surface by a wire or (in Du Bois-Reymond's arrangement) a bridge of filter paper or clay soaked in salt-solution. In this experiment what is really done is suddenly and greatly to change the resistance in the external part of the circuit through which the nerve-current is flowing; this of course is equivalent to suddenly increasing or decreasing the flow at all points of the circuit; so that the effect of suddenly applying or withdrawing the external conductor is really that of cross-circuiting or the reverse, *i.e.*, is the same as if the demarcation-current were to be suddenly closed or opened. The maximal distance which may separate the points of contact at cut and longitudinal surfaces without annulling the stimulating action of the demarcation-current may thus be regarded as closely corresponding with the maximal distance along which the demarcation-current could make itself felt as stimulus if it were suddenly to come into existence or disappear. Now the normal effect of excitation is to produce rapidly a local negativity like that at the cut surface; the electrical effects at neighboring regions of the nerve must therefore be the same as in the case just considered. Hence we infer that the action-current, on its sudden local appearance following stimulation, itself stimulates electrically the nerve at a distance from the locus of excitation at least equal to the maximal distance through which the demarcation-current may stimulate in the above experiment, — *i.e.*, from 2 to 3 centimeters. In the intact animal the maximal distance through which the stimulating action extends is probably greater than this, since in the excised nerve the conditions are necessarily more or less abnormal, and local potential-differences at the cut ends of side branches or at points of injury will give rise to minor demarcation-currents which will interfere with and partially compensate the action-current, thus decreasing its stimulating effect. It is well known that in the experiment where the nerve is stimulated by its own demarcation-current as the result of being dropped suddenly on another conducting surface (a piece of tissue or a pad of clay

zungsberichte d. kais. Akad. Wiss. Wien, math.-naturw. Kl., 1882, lxxx$_V$, Abth. 3, p. 240.

moistened with salt-solution), it is highly important that the latter should be "isoelectric," since otherwise interferences arise which impair the success of the experiment. In the above experiment with the demarcation-current the external conditions are always more or less unfavorable to stimulation by weak currents, and we may infer that in the intact animal the action-current, when it arises at any point in the nerve, exerts a stimulating effect at a greater distance from this point than the maximal distance separating the electrodes in Du Bois-Reymond's experiment. How much greater is difficult to say on *a priori* grounds, and any direct experimental determination seems impracticable.

Another indirect method of attacking the present problem is to determine the maximal distance that may separate electrodes having the same potential-difference as that between active and inactive regions (20 to 30 millivolts in nerve) without preventing stimulation; *i.e.*, to determine the potential-difference between electrodes a known distance apart when the current has an intensity just sufficient for stimulation. We may in fact regard excited region and unexcited region as two areas of different potential united by a conductor. The current flowing between these regions, if sufficient in intensity and duration, will excite the nerve along the line of flow. The stimulating effect will be the same as if the two regions were electrodes differing in potential by a certain degree.

Recently I have made a large number of determinations of the least potential-difference required for stimulating the frog's sciatic nerve through platinum electrodes separated by a stretch of nerve varying from 1 to 2 centimeters in length. These experiments have shown that while the sensitivity of the nerve to electrical stimulation varies considerably in different nerve-muscle preparations, yet in sensitive nerves currents of 10 to 15 millivolts P.D. frequently cause well-marked stimulation with the electrodes 15 or 20 millimeters apart, and not infrequently stimulation occurs with much weaker currents.

The following is an instance of an experiment with a somewhat more than usually sensitive pair of nerves. The electrodes were platinum wires of about 0.3 mm. diameter; these were connected through a rocking key or pole-changer to the zero post and slider

of an Ostwald meter-wire rheocord. The rocking key was also connected with a Weston millivolt meter, so that (in the absence of the cross wires) the E.M.F. of the current supplying the electrodes could be read off directly by turning the rocker. The rheocord was connected with a single Edison normal cell. The nerve was suspended between electrodes 15 millimeters apart.

The following figures give the potential difference (millivolts between the electrodes) of the weakest current that caused definite contraction of the leg or foot muscles on make or break of the current. Both sciatic nerves of the frog (*Rana virescens*) were used with both ascending and descending currents.

	Direction of current	Voltage of weakest current
Nerve 1	Descending	10 mv. (stimulation at make, not at break)
"	Ascending	13 mv. (stimulation at break, not at make)
Nerve 2	Descending	9 mv. (stimulation at make only)
"	Ascending	10 mv. (stimulation at break only)

The following is a record of a similar experiment with a less sensitive pair of nerves.

	Direction of current	Voltage of weakest current
Nerve 1	Descending	22 mv. (stimulation at make only)
"	Ascending	28 mv. (stimulation at break only)
Nerve 2	Descending	20 mv. (stimulation at make only)
"	Ascending	22 mv. (stimulation at break only)

Preparations are not infrequently more sensitive than either of the above, and occasionally stimulation occurs with a P.D. of only 5 or 6 millivolts. This extreme sensitivity is most frequently met after the nerve has been exposed to the air for a time and has begun slightly to dry. Usually such marked increase of irritability is followed by spontaneous twitching of the muscle; bathing the nerve in Ringer's solution then arrests the twitching.

With the electrodes 2 centimeters apart the minimal voltage is somewhat higher, but is frequently so low as 10 to 15 millivolts, and in some cases I have obtained stimulation with 8 millivolts.

It has been my experience that the make and the break of these weak constant currents are almost equally effective as stimuli. All that appears to be needed is the rapid establishment of a certain minimal potential-gradient along the nerve. The above observations show that this gradient may be so low as 10 millivolts in 2 centimeters, which is the same as 30 millivolts (the approximate variation of potential in the action-current) in 6 centimeters. It might possibly be objected that the nerve was in a hypersensitive condition in these experiments, or that the observed stimulation was really the result of a summation of the effects of the external current and of local nerve-currents due to drying or injury. Observation shows, however, that the voltage of the minimal exciting current tends toward an approximately constant lower limit when the electrodes are a definite distance apart, — a fact indicating the existence of a definite threshold-intensity for a current traversing the nerve. This intensity is not greater than that of a current flowing between two regions of a nerve several centimeters apart differing in potential by 20 or 30 millivolts, the approximate E.M.F. of the action-current of frog's nerve.

We conclude that the current flowing between excited and unexcited regions of a normal frog's motor nerve is sufficiently intense at a distance at least $2\frac{1}{2}$ or 3 centimeters from the excited region to cause stimulation at that point.

The question of whether its duration is sufficient may readily be answered, since we have recent accurate determinations by Lapicque and Lucas of the least effective duration of currents of different strengths, — ranging from the weakest currents that will excite with any duration of flow, to currents whose least effective duration is a small fraction of that of the weakest exciting current. Within the range of conditions to which Nernst's rule applies, the current-strength required for stimulation varies approximately inversely as the square root of the duration. From this it is evident that a current of briefer duration than the rising phase of the action-current (*ca.* 0.001 second at 20°) may stimulate a nerve if its intensity is sufficient. The question is, whether the current flowing between the region already active and an inactive region 2 or 3 centimeters distant is sufficiently intense to stimulate with a duration not greater than that of the rising phase of the action-

current. Lapicque has shown that the minimal current that will stimulate a nerve with infinite duration of flow requires for stimulation a duration not much greater than that of the rising phase of the action-current; and in order to stimulate with a duration not greater than this (*i.e.*, *ca.* .001 sec.) the intensity needs to be increased to only a relatively slight degree (respectively 33 per cent and 50 per cent for the two experiments at 24.5° and 18° which he cites).[52] This general rule holds true not only of nerve but of other irritable tissues, and probably of irritable tissues in general.[53] The time occupied by the rising phase of the action-current is in fact closely similar to the time during which a current of threshold-intensity requires to flow in order to stimulate. This time is characteristic for any irritable tissue, and constitutes what Lapicque calls the chronological factor in electrical excitation.[54] It varies from tissue to tissue in a manner which shows close parallelism with the characteristic time-relations of the bioelectric variation, its exact duration in any tissue being closely similar to — though apparently usually somewhat greater than — that of the rising phase of the electrical variation.[55] It is thus clear that the action-current lasts quite sufficiently long to stimulate the adjoining areas of the irritable tissue at a distance not much less than the maximal distance at which (on the above estimate) its intensity is sufficient.

In order to simplify our conception of the conditions as much as possible, let us regard the region actually undergoing excitation as stationary; it is evident that the electrical effect at any point

[52] *Cf.* LAPICQUE: Journale de physiologie normale et pathologique, 1907, ix, p. 628; *ibid.*, 1908, x, p. 599. The conditions are the same at lower temperatures; here, of course, the duration of the action-current is proportionately prolonged.

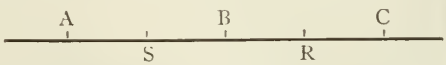
[53] *Cf.* the observations of LUCAS on the frog's sartorius, Journal of Physiology, 1908, xxxvii, p. 475, and of LUCAS and MINES on the sciatic and sartorius of the toad, *ibid.*, 1907, xxxvi, p. 334.

[54] *Cf.* LAPICQUE: Journale de physiologie, 1908, x, p. 601.

[55] *Cf.* LAPICQUE, *loc. cit.;* also *Cf.* LUCAS: Journal of Physiology, 1910, xxxix, p. 461. According to Lucas the normal rate of change characteristic of the excitatory process in a particular tissue is what determines its time-factor of electrical stimulation. Hence this is brief in rapidly reacting tissues like nerve, etc.

beyond this active area will vary in intensity during the time occupied by the entire negative variation at the excited region, and that it will have the requisite intensity for stimulation at that point during only part of the time occupied by the whole variation. If we consider the intensity alone, it is clear that the effect will "reach" farthest — *i.e.*, will be sufficient at a certain distant point as regards mere intensity — when the action-current curve is at its apex; but the *duration* of the branch of the action-current traversing this most distant point will probably be insufficient for excitation. There will, however, be an intermediate point or region where the current is just intense enough and lasts just long enough to stimulate; this region will then at once become the site of a new excitation-process which will produce similar effects at an equidistant point beyond. If the distance of this point from the excitation-area is s centimeters, and the time required for the local excitation-process to reach the required stage for excitation at this point is t seconds, the state of excitation will be propagated with the velocity s/t centimeters per second. With $s=3$ centimeters and $t=.001$ second, the propagation-velocity will be 30 meters per second.

Naturally the progress of the excitation-wave is continuous and does not occur on a succession of leaps, as the above simplified manner of conceiving the phenomenon would seem to imply. This apparent implication, however, merely results from a neglect to consider the processes at other than the two points S, considered as the stimulating point of the nerve, and R, considered as the responding point. Needless to say, the region actually undergoing the changes accompanying excitation occupies a considerable length of the nerve (*ca.* 30 mm. in a single impulse). The actual process would be better described as follows: traveling in advance of the region already active, A B, there is a region B C as yet



inactive but through which a branch of the action-current is flowing; at a certain critical distance B R beyond the active region this current will have the intensity, duration, and rate of development necessary to stimulate the nerve at that point (R). The

greater this critical distance, and the more rapid the local excitation-process, the more rapid will be the rate of propagation.

It is necessary also to consider whether the rate of change of this branch of current is sufficient for excitation. The action-current does not appear suddenly, but rises continuously to a maximum and subsides at a rate that varies characteristically from tissue to tissue. Any stimulating current must not only have a sufficient intensity and duration, but must rise to its stimulating value with sufficient rapidity. The rate of change of the action-current in nerve is, however, actually such that its stimulating efficiency is little if any less than it would be if the current were to rise to its maximum instantaneously. This is shown by Lucas' experiments in the sciatic nerve of the toad, which show that a current which takes .03 seconds to rise to its maximum requires to be only about 20 per cent stronger than an instantaneous current of the same stimulating value.[56] Although the time-factor of excitation in the frog's nerve is somewhat shorter than in the toad's nerve, there is no doubt that a current rising to its maximum in a period 20 to 30 times briefer would have essentially the same effect as an instantaneous current. The rate of change of the stimulating current is related to that of the action-current of the tissue in the same manner as the duration of the least exciting current is related to that of the action-current.[57] A tissue with a brief time-factor of excitation requires a rapidly changing current for stimulation, and *vice versa*. We conclude, therefore, that the rate of change of the action-current in any tissue is adequate for the stimulation of that tissue. The slowly rising electrical variation of a slowly responding tissue is amply capable of stimulating that tissue; although it may be unable to stimulate another tissue with a more rapid rate of response.

We may summarize the foregoing briefly as follows: the facts and considerations adduced indicate that at some distance (probably about 3 centimeters in frog's nerve) from the region already in a state of excitation the branch of the action-current traversing the tissue is sufficient in intensity, duration, and rate of change to stimulate the tissue at that point and hence to initiate a new state

[56] Lucas: Journal of Physiology, 1907, xxxvi, p. 253.

[57] *Cf.* Lapicque: *loc. cit.;* Lucas: *loc. cit.*, 1908.

of excitation; this in its turn originates a similar excitation at an equidistant point beyond, and so on. Thus the excitation-state is self-propagating by reason of the electrical variation by which it is accompanied, and the rate at which this electrical variation rises to its maximum is the chief factor in determining the velocity of conduction.

This view accounts not only for the propagation of the excitation-wave and for the varying rate of propagation in different tissues, but also for certain other hitherto unexplained peculiarities of irritable tissues. As already pointed out, it affords a possible basis of explanation for irreciprocality of conduction, if it is assumed that the time-factors of excitation of the contiguous neurones are sufficiently different. It also assists in explaining Carlson's generalization that rapidly contracting muscles typically have rapidly conducting nerves, and *vice versa*.[58] In this case we have an instance of transmission of the excitation-state from one irritable element, the nerve, to another, the muscle.[59] On the present hypothesis the muscle cell is stimulated by the electrical variation of the nerve where it branches over the surface of the muscle at the end-plate.[60] The process of transmission from element to element is thus of the same nature as from one region of a continuous element (*e.g.*, nerve) to another. We have seen that for stimulation of the adjoining inactive region it is necessary that the electrical current passing between the active region A B and the inactive B C should have a certain minimal intensity and duration. The intensity is a function of the distance — *i.e.*, electrical resistance — between the two regions; the duration depends on the duration of the local electrical variation at region A B, — or more

[58] Carlson: *loc. cit.*

[59] The existence in some cases of an intermediary element, the nerve end-plate, with a characteristic excitation-rate of its own, does not essentially alter the conditions.

[60] This view recalls the "Entladungshypothese" put forward by Krause and Kühne, in 1863 and 1864 (*Cf.* Hermann's Handbuch, i, p. 258), and later criticized, though accepted in a modified form, by Du Bois-Reymond. The latter favored the view that the negative variation of the nerve directly stimulated the muscle; this view Hermann regards as identical with his own view (first put forward in 1872) that the electrical variation is the basis of propagation.

precisely on the duration of that portion of it which has reached a sufficient intensity to affect region B C. If this duration is insufficient, region B C will not be affected and the impulse will fail to be propagated. The same considerations apply to transmission of excitation between separate irritable elements. The action-current of the exciting element must have a duration sufficient to enable it to excite the responding element; it must also rise to its maximum with sufficient rapidity. Now rapid nerve-conduction means rapid local electrical variation, *i.e.*, brief duration of the current-strength requisite for stimulation. Only a rapidly responding muscle can thus be stimulated by the action-current of a rapidly conducting nerve; in the case of a slowly responding muscle the duration of the action-current of such a nerve would be insufficient to cause excitation. Similarly a slowly conducting nerve is unable to impart the requisite stimulus to a rapidly responding muscle, because the electrical variation in such a nerve, even if sufficiently intense, rises to its maximum too gradually to stimulate a tissue with a brief time-factor of excitation, although it might well be capable of stimulating one with a slower rate of response.[61] The correlation between the velocity of conduction and the velocity of contraction is thus not to be regarded simply as an adaptive arrangement which aims at securing quickness of reaction by shortening the total time of response, but as essentially an expression of the general validity of the fundamental laws of electrical excitation, namely, that the stimulating (*i.e.*, polarizing) current must have a certain minimal duration and a certain minimal rate of development as well as a certain intensity. A slowly conducting nerve is by its very nature one whose action-current is unable to stimulate a rapidly responding tissue, and *vice versa*.[62]

The chief objection to the present hypothesis seems to me to be that transmission may be blocked by local narcotization, or by mechanical constriction or local injury which affects only a short stretch of nerve. Such local alteration of course leaves electrical

[61] Compare with the examples of failure of secondary excitation quoted above from KÜHNE p. 424.

[62] Conceivably, of course, intermediaries — "transformers" — might exist which would render such transmission possible, but this possibility need not be considered here.

conductivity unimpaired. The conditions of many of these blocking effects are, however, imperfectly understood at present, and it does not appear that their existence is necessarily incompatible with the present view. In the case of ligature, severing, or the local application of a poison, there is local injury; this involves the production of demarcation-currents extending in both directions from the region of injury for some distance through the adjoining tissue; these currents not only exert electrotonic influence, but they may directly compensate the action-current of an approaching excitation-wave and so prevent its effect from extending beyond the point of injury.[63] The reversible blocking action of narcotics is more difficult to explain. The narcotized region must, however, have a certain length. Recent experiment shows that the extinction of the excitation-wave on entering a narcotized area is not an immediate, but a more or less gradual process. The excitation-wave may pass such a region if the latter is not too long.[64] Its length may, however, be considerably less than the 2 or 3 centimeters across which the electrical impulse from an active region is above supposed to be effective. It is possible here also that demarcation-currents interfere with the action-current and prevent its transmission across the narcotized area. I shall not attempt to discuss this difficulty further at present. The possibility that the

[63] It is known that pricking the *His* bundle so as to injure directly only a few fibres may completely block for a time the impulse from auricles to ventricles. For instances *cf.* Erlanger and Blackmann: Heart, 1910, i, pp. 211, 214, 217. Similarly, partly cutting across a bridge of muscle (sufficiently wide to conduct regularly) connecting two portions of the turtle's ventricle may cause complete temporary block. This disappears in time on bathing in Ringer's solution. Then a further injury at or *near* the original cut will restore the block. (Observations of Dr. W. E. Garrey; similar observations are contained in his article on fibrillation in the American Journal of Physiology, 1914, xxxiii, p. 397.) Some influence due to the injury of adjoining elements prevents the transmission of impulses along the uninjured elements. Garrey has also expressed the view that changes in potential at the site of block form a chief factor in the production of this effect (public lecture delivered at Woods Hole, July, 1913).

[64] *Cf.* Adrian: Journal of Physiology, 1912, xlv, p. 389. Also the papers of Fröhlich (Zeitschrift f. allg. Physiol. 1904, iii, p. 148) and others, showing a progressive decrease of impulses in narcotized regions or others conducting with a "decrement."

action-current is the actual normal basis of transmission, even in nerve, seems on the whole strongly favored by the facts and considerations cited above. That there are difficulties in extending this view in detail to special cases of transmission it would be useless to deny. But it is only by definitely formulating an hypothesis and tracing its consequences that we can decide as to its adequacy or inadequacy. Criticism and further research both are necessary before a final decision can be reached.

It is evident that according to the point of view urged in this paper the problem of physiological conduction ceases to be a special problem, and becomes a subordinate part of the general problem of stimulation, — more specifically of electrical stimulation. The idea that conduction and excitation are two separate and independently variable properties of an irritable tissue has shown itself inconsistent with the course of recent research and is now very generally rejected.[65] Why the electrical current should stimulate is the essential problem. The view that electrical stimulation is an expression or consequence of the production of an electrical polarization at the surfaces of the irritable elements was frequently urged by Hermann,[66] and has been placed on a firm footing by the work of Nernst and his successors. A further important step was made when Brünings [67] first pointed out what seems to be the essential implication of the law of polar stimulation, that the necessary antecedent for stimulation is a *depolarization* of the plasma membrane, *i.e.*, a polarization-effect in the inverse direction to the pre-existing or physiological polarization and hence having the effect of partly compensating this. The electrical current thus stimulates when it suddenly *decreases* the physiological polarization to a certain critical degree. To do this it must act for a certain time and rise to its maximum with sufficient velocity.

[65] *Cf.* Fröhlich: *loc. cit.;* also the discussion by Lucas: Croonian Lecture, *loc. cit.;* and Verworn: "Irritability," Yale University Press, 1913, chap. vi.

[66] *Cf.* Hermann: Handbuch, Vol. ii, 1, p. 193 and elsewhere.

[67] *Cf.* Brünings: Archiv f. d. gesammte Physiologie, 1903, *c*, p. 367. Hermann entertained similar views: *viz.*, that in nerve the stimulating action had its seat at the boundary of axis cylinder and sheath, and that a fibre is stimulated when the positive polarization of its longitudinal surface is decreased or its negative increased (*loc. cit.*, p. 193).

The essential question thus becomes: why should a slight decrease in the electrical polarization of the plasma membrane cause stimulation? Evidence from many sides indicates that during stimulation the plasma membrane temporarily loses its semi-permeability;[68] and since slight depolarization results in stimulation, the inference seems justified that the normal resting permeability of the membrane *depends* on its electrical polarization.[69] The view that the permeability of the plasma membrane is a function of the electrical polarization has also been independently urged by several other investigators.[70] The nature of the relation between the electrical polarization and the other properties of the membrane is still uncertain. During the normal resting condition of the irritable element the membrane appears to retain its semi-permeability unaltered; a sudden depolarization results in a change which has the effect of temporarily altering both its osmotic and its electromotor properties. Stimulation is apparently an expression or consequence of this change. At present the evidence indicates (1) that the change in permeability associated with stimulation is not a direct effect due to merely physical changes in the protoplasmic surface-layer, but is the consequence of a chemical reaction which alters the character of the surface-film and temporarily deprives it of its normal semi-permeable and electromotor properties, and (2) that this chemical process may in a highly irritable tissue like nerve be initiated by any slight local decrease in polarization provided the change is sufficiently rapid. That the process which is immediately initiated or released by the polarization-change is a *chemical* one is indicated both by the high temperature-coefficient of the stimulation-process, and by the wide variation which its

[68] I have summarized and discussed this evidence in various places: *Cf.* American Journal of Physiology, 1909, xxiv, p. 14; 1911, xxviii, p. 197; Science, 1909, p. 245; Biological Bulletin, 1909, xvii, p. 188.

[69] *Cf.* American Journal of Physiology, 1908, xxii, p. 79; Biological Bulletin, *loc. cit.*

[70] *Cf.* Girard: Journal de physiol. et pathol. gén., 1910, xii, p. 471; W. B. Hardy and H. W. Harvey: Proc. Roy. Soc., Ser. B. 1911, lxxxiv, p. 217; Höber: Physikalische Chemie d. Zelle u. d. Gewebe (3d Ed.), 1911, p. 308 *seq.;* Mines: Journal of Physiology, 1910, xl, p. 309, 1912, xliii, p. 467; A. J. Clark: *ibid.*, xlvii, p. 103.

velocity shows in different tissues.[71] The conditions suggest that the normal polarization is what prevents certain constituents of the surface-layer from interacting, some critical reaction being held in check so long as the polarization has a certain value — very much as the chemical interaction between plates and liquid in a battery with open circuit is held in check by the polarization at the surface of the plates. The sensitivity of highly irritable elements to variations in the electrical polarization of their surface-films is difficult to understand on any other hypothesis known to me at present.[72]

Summary

1. Numerous instances are cited from the literature showing the existence of a general parallelism between the rate at which the action-current curve rises to its maximum in any irritable tissue and the rate of propagation of the excitation-wave in that tissue.

2. Various other facts are cited favoring the hypothesis that the transmission of excitation from one region of an irritable tissue to another is directly due to electrical excitation of the inactive region, at some distance from the active region, by means of the portion of the action-current flowing between the two regions.

3. On this view the rate of propagation depends on two factors: (1) the time required for the action-current to rise to its full intensity after stimulation, and (2) the maximal distance from the active region at which the intensity and duration of the branch of current traversing the inactive region are sufficient for stimulation.

[71] Estimates of the relative velocities of the excitation-process in different irritable elements are given in the paper by Lucas; Journal of Physiology, 1910, xl, p. 225; also by Lapicque: Comptes rendus de la Société de Biologie, 1905, lvii, p. 503.

[72] Changes in the polarization of the plasma membrane must involve changes in the aggregation-state of the protoplasmic colloids (*cf.* my earlier paper *loc. cit.*, 1908), since, as is well known, colloids are sensitive to changes in the electrical polarization of the colloidal particles. The possibility that the primary change in stimulation may be one of this kind should thus also be considered. Undoubtedly some change in the aggregation-state of the colloids of the membrane is involved in stimulation, but whether this is primary or secondary cannot be said at present.

If the time occupied by the rise of the action-current to the critical stimulating point is t seconds and the maximal distance at which it causes stimulation is s centimeters, the velocity of propagation will be s/t centimeters per second.

4. In frog's nerve the distance s is estimated at from 2 to 3 centimeters and the time t as .001 second (the duration of the rising phase of the bioelectric variation at 18°); this corresponds to a velocity of 20 to 30 meters per second at this temperature. Increase of propagation-velocity with rise of temperature appears to be due chiefly to the increased rate of rise of the action-current; at 38°, with $Q_{10} = 2$, it would be 80 to 120 meters per second.

NOTE UPON THE MOVEMENTS OF THE INTESTINAL VILLI

By B. F. HAMBLETON

[*From the Physiological Laboratory, Medical Department, Vanderbilt University, Nashville, Tenn.*]

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THE structure of the villi of the small intestine is such that one would naturally infer that they possess the property of motion. Accordingly observations were begun on the living animal to see if such is the case. The dog was selected for these experiments.

The technique employed in performing the experiments is as follows:

The dog is anaesthetized with ether and the abdomen opened in the median line. A loop of the small intestine is picked up and passed through a slit in a thin board, or a slab of hard paraffin. The intestine is opened longitudinally for a distance of two or three inches opposite to the attachment of the mesentery and spread out and fastened to the board by means of tacks inserted into the board on each side of the slit, care being taken that the mesentery is not stretched nor pressed upon so as to interfere with the circulation or nerve supply.

The observations are then made by means of the binocular microscope, using a magnification of from 23 to 61 diameters. In dogs that have been fasting from 24 to 48 hours the villi are generally found extended, fallen over in various directions, and covered with a mucus-like substance. Local application of a few drops of any of the following solutions: — peptone, glucose, weak alkali solutions (sodium carbonate), dog's bile, diluted, physiological salt solution, or distilled water, is followed in a few seconds by the villi rising and beginning lashing-like movements in various directions. Very soon a second distinct movement begins which

consists of an alternating shortening or retraction, and extension of various villi in the field. These movements are independent of peristalsis. The application of hydrochloric acid, $\frac{1}{10}$ per cent solution, checks the movements, and the villi return to the resting condition, i.e., fall over and soon become covered with mucus. A 10 per cent alcoholic solution first stimulates, then depresses, and soon stops the movements entirely. The addition of water, or saline solution, 0.9 per cent, restores activity and the villi begin movements again. Intravenous injection of nicotine (1 mg. to 2 mg. per kilo) first stimulates, then depresses the movements, particularly that of retraction and of extension. Local application of solutions that caused active movements before the injection of the nicotine now have no effect. Atropine (1 mg. per kilo, intravenously) stops the movements. Local application of glucose, peptone, or other solutions that stimulated previous to the use of the atropine cause the lashing-like movement to return, but there is no retraction and extension.

From the observations thus far made, which have been quite constant on all animals examined, we reach the following conclusions:

First. That the villi possess distinct movements that are independent of peristalsis.

Second. That these movements are of two kinds: (1) A lashing movement, which may be supposed to aid in the mixing of the intestinal contents and thus promote the action of the digestive secretions as well as the process of absorption. This movement is not stopped by atropine. (2) An alternating retraction and extension, a form of movement which may be of special value in the act of absorption, particularly the absorption through the lacteals. This movement is abolished by both nicotine and atropine and is therefore under the control of the peripheral nervous mechanism. It may be regarded, probably, as a local reflex. Further investigation is necessary to determine whether the central nervous system is involved in this reflex.

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