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THE  
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DECEMBER 1, 1905.

NO. I.

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STUDIES IN THE PHYSIOLOGY OF MUSCLE.  
I. OBSERVATIONS ON THE TONUS OF HEART MUSCLE.

BY W. T. PORTER.

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

AT the fifteenth annual meeting of the American Physiological Society, held in Washington, D. C., Dec. 30, 1902, the writer presented graphic records demonstrating the following facts: "1. As the tonus increases, the conductivity of the heart muscle diminishes. 2. The height of the tonus contraction is proportional to the strength of the stimulus; the law of "all or none" does not apply. 3. The tonus contraction has no refractory period; extra contractions (both of tonus form and fundamental form) may be produced during any phase of tonus contraction. 4. Tonus contractions may be superposed as contractions of skeletal muscles are superposed."<sup>1</sup>

The publication of the curves shown to the Physiological Society and the full report of the experiments there presented have been deferred in order to permit the collection of additional data. In discussing the material thus secured it will be convenient to begin with the original observations.

METHOD.

In the following observations the extirpated heart of the tortoise was fastened to a paraffined cork by a U-shaped wire placed across the auriculo-ventricular junction. The cork bearing the heart was fixed in a clamp in a moist chamber and a delicate hook passed through

<sup>1</sup> W. T. PORTER: This journal, 1902-03, viii, p. xxvi.

the auricle. A very fine copper wire which was wound about the free end of the hook transmitted the contractions to a recording lever, the moving parts of which weighed about 0.4 gram. The hook also served as one electrode, the other being the U-shaped wire with which the heart was fastened. Wires led from these to the secondary coil of a Kronecker standard inductorium. Unless otherwise mentioned, break induction currents were employed as stimuli.

#### AS TONUS INCREASES, CONDUCTIVITY DIMINISHES.

**Conductivity for the fundamental excitation.**—Alterations in conductivity were determined by measuring the duration of the mechanical latent period of extra fundamental contractions produced during different degrees of tonus. The time was recorded by the author's magnetic signal connected with a Zimmermann tuning-fork electrically driven at one hundred double vibrations per second. Another magnet, which was placed in the primary circuit of the inductorium, recorded the moment of stimulation. At the beginning of the experiment the writing point of this second magnet was carefully set in the line drawn by the heart lever at rest, so that the line drawn by the writing point served also as a base line from which could be measured the variation in tonus. The heart contracted with its usual rhythm superposed upon slower tonus contractions. The auricle was stimulated with break induction currents in the interval between two spontaneous tonus contractions. An extra fundamental contraction was thereby produced. The interval between the moment of stimulation and the beginning of this extra contraction was recorded. As the tonus curve drawn by the heart lever rose or fell with reference to its original position or base line, the stimulation was repeated. Thus a comparison was made between the mechanical latent periods of the fundamental contractions when the heart was in widely different stages of tonic contraction. Following is a typical protocol.

*Experiment, Nov. 11, 1902.* The heart of a tortoise was fastened to a cork by a U-shaped wire placed across the auriculo-ventricular junction and the preparation placed in a moist chamber. The movements of the right auricle were recorded by a writing lever attached to a hook passed through the tip of the auricle as described above. Extra fundamental contractions were obtained by break currents from a Kronecker inductorium supplied by two Daniell cells. A signal magnet placed in the primary circuit marked the moment of stimulation. The time was re-

corded by a signal magnet connected with a tuning-fork giving one hundred double vibrations per second. The variation in the mechanical latent period is shown in Table I, in which are given the time of the observation, the distance of the writing point above (+) or below (−) the base line, and the number of hundredth seconds between the moment of stimulation and the beginning of the consequent fundamental extra contraction.

It is obvious from Table I and Fig. 1 that the conductivity for the fundamental contraction lessens as the tonus increases. This conclu-

TABLE I.

Time at which heart muscle was stimulated.	Amount of tonus, <i>i. e.</i> , distance of writing point above or below base line.	Conductivity measured by duration of latent period.
a.m.	m.m.	0.01 sec.
10.30	− 1 +20	10 18
10.45	+10 − 2	12 6
10.50	+18 − 2	13 6
11.00	− 5 +25	8 21
11.05 <sup>1</sup>	− 4 +14	10 15
11.10	− 7 + 3	10 14
11.30	−12 +19	10 18
11.35	−12 + 6	11 14
11.45	−11 + 7	8 12

<sup>1</sup> The observation made at 11.05 is shown in Fig. 1.

sion was confirmed by a large number of measurements made by Dr. Lamb and the writer<sup>1</sup> in 1904, undertaken to determine quantitatively the relation between the conductivity and the tonic contraction. These measurements will be discussed in a subsequent paper of this series.

<sup>1</sup> W. T. PORTER and F. H. LAMB: This journal, 1905, xiii, p. xxiii.

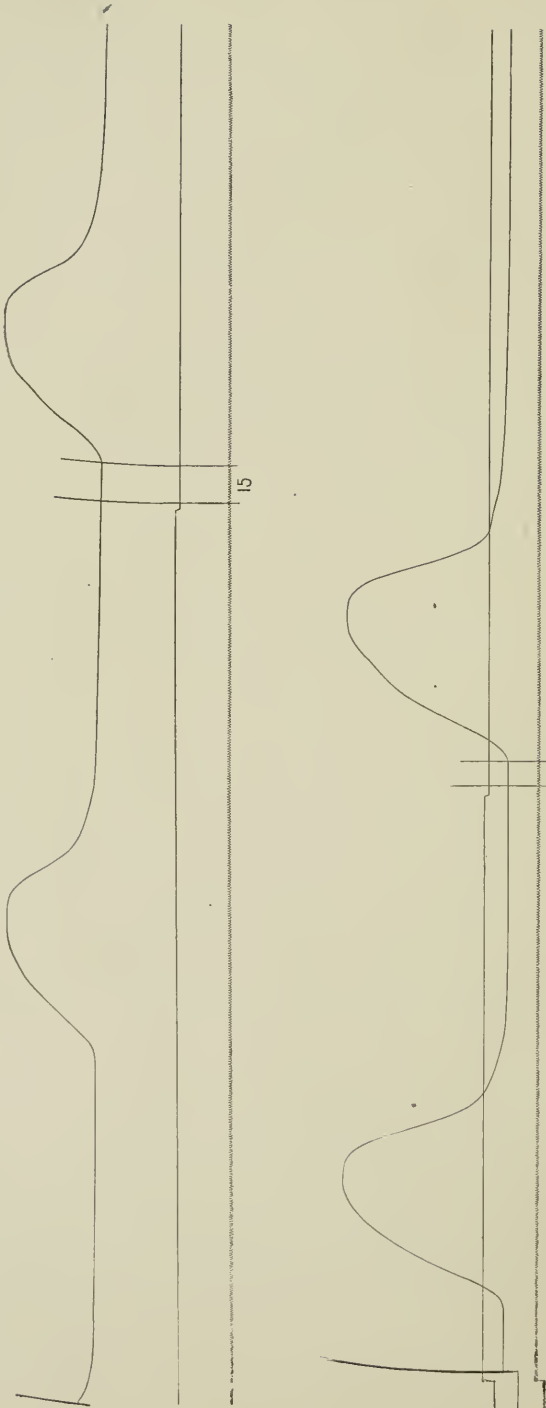


FIGURE 1.—A spontaneous and an extra fundamental contraction of the tortoise auricle, recorded when the tonus is low (lower curve) and when the tonus is high (upper curve). With low tonus, the latent period is 10 hundredths second; with high tonus, 15 hundredths. The conduction diminishes as the tonus increases.

Further confirmation is afforded by experiments made in July, 1905, on the relation between tonus and conductivity in the intestine of mammals.<sup>1</sup> A ring of intestinal muscle in the middle of a long piece of extirpated but surviving intestine can easily be thrown into a tonic contraction which far outlasts the stimulus. A peristaltic wave leaving either end of the intestinal tube is delayed or wholly arrested on reaching the tonus ring. Indeed the peristaltic wave often turns at the tonus ring and moves by antiperistalsis back to the end of the tube.

These several observations strengthen the explanation of fibrillar contraction suggested by the writer<sup>2</sup> in 1893 and 1899. Fibrillar contraction of the heart, according to this hypothesis, may be due to an interruption of the contraction wave. The tonus of the ventricle is greatly increased at the onset of fibrillation (1899), and co-ordinated beats do not return unless the extreme tonic contraction is considerably lessened. The appearance of tonic spasm at several points breaks up the normal conduction of the contraction wave, by interposing here and there regions the intense tonic contraction of which blocks the passage of the contraction wave and leaves the remaining muscle fibres dissociated and in confusion.

In 1898 the writer<sup>3</sup> demonstrated the compression of the intramural branches of the coronary vessels by the contraction of the muscular fibres around them,<sup>4</sup> and pointed out that the volume of blood passing through the coronary vessels is increased by an increase in either the force or the frequency of the heart beat, probably because of the periodical emptying of the intramural vessels by the systolic squeeze of the surrounding fibres. To the influence of the fundamental contractions on the intramural blood supply of the heart may now be added the influence of tonus contractions. Observations made in this laboratory<sup>5</sup> have shown how quickly the heart responds to the diminution of its blood supply, especially when the supply is

<sup>1</sup> PORTER, LAWRENCE, and NEWBURGH: To be presented to the American Physiological Society in December, 1905.

<sup>2</sup> W. T. PORTER: *Journal of physiology*, 1893-1894, xv, p. 134; *This journal*, 1899, ii, p. 129; *Ibid.*, 1901-1902, vi, p. xxiv; *Ibid.*, 1903, viii, p. xxvi; *Ibid.*, 1905, xiii, pp. xxiii and xxiv.

<sup>3</sup> W. T. PORTER: *This journal*, 1898, i, pp. 145-163.

<sup>4</sup> First studied by REBATEL: *Recherches expérimentales sur la circulation dans les artères coronaires*. Paris, 1872.

<sup>5</sup> W. T. PORTER: *Journal of experimental medicine*, 1896, i, pp. 46-70; MAGRATH and KENNEDY: *Ibid.*, 1897, ii, pp. 13-34; PORTER: *This journal*, 1898, i, pp. 71-82.

limited. Compression of the intramural vessels by prolonged tonic contraction of the enveloping muscle (Fig. 6) might easily lead to fatal arrest of the heart in cases in which the blood supply was already small, as in arterio-sclerosis of the larger coronary branches.

**Conductivity for the tonus contraction.**—It has just been shown that the conductivity for the fundamental excitation is lessened as the tonus increases. It will now be necessary to inquire whether the conductivity for the tonus excitation is also lessened by an increase in tonus. In other words, is the interval between adequate stimulation and the consequent tonus contraction greater when the tonus preceding stimulation is high than when the tonus is low?

A careful search throughout my curves fails to show any relation between the conductivity for the tonus excitation and the preceding state of tonus contraction. Thus, in Fig. 7, the first stimulus was given while the tonus was low; the second and third while the tonus was high; yet the latent period was practically the same in all three. I am therefore obliged to conclude that variations in tonus do not alter the conductivity for the tonus excitation. This is evidently a negative conclusion, and it is much to be regretted that a decision the theoretical interest of which is undeniable should be based on negative results.

#### THE HEIGHT OF TONUS CONTRACTION IS PROPORTIONAL TO THE STRENGTH OF THE STIMULUS.

The tonus contraction is proportional to the strength of the stimulus, as is shown by Figs. 2 and 3, recorded Nov. 26, 1902. The protocol follows.

*Experiment, Nov. 26, 1902.*—The contractions of the right auricle of the tortoise heart were recorded as described above. An Ewald interrupter was placed in the primary circuit of a Kronecker standard inductorium supplied by twelve Daniell cells.<sup>1</sup> In every phase of tonus contraction, both fundamental and tonus contractions could be produced by electrical stimulation. The stimulus employed for the tonus contractions was a series of five induction currents, the interval between successive induction currents being a little less than one second. The secondary coil was placed at 2000 of the Kronecker scale. The intensity of the stimulus was then increased from 2000 to 3000, 4000, and 5000. The height of the tonus contraction increased with each increase of stimulus, but the fundamental contractions were partially or completely obscured (Fig. 2). High

<sup>1</sup> This large number of cells was necessary to drive the Ewald interrupter.



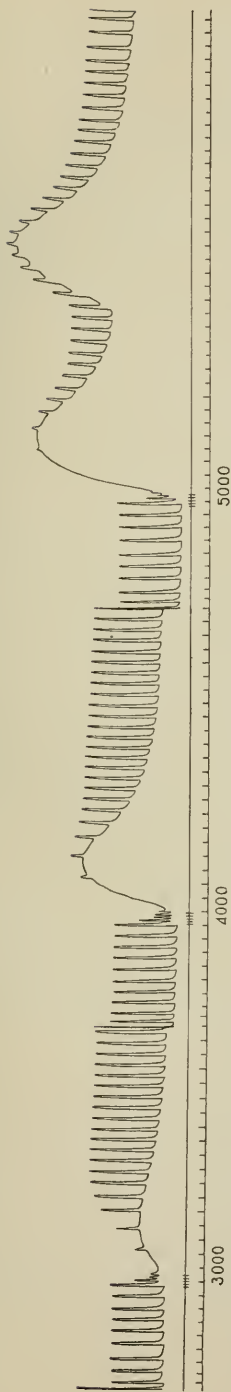


FIGURE 2. — Tonus curves from the auricle of the tortoise. The contraction is proportional to the intensity of the stimulus. The stimuli were the groups of break currents indicated on the middle line. For the first stimulus the secondary coil was at 3000 of the Kronecker scale, for the second at 4000, and for the third at 5000. Note the inhibition of the fundamental contractions. The lowest curve records every fifth second.

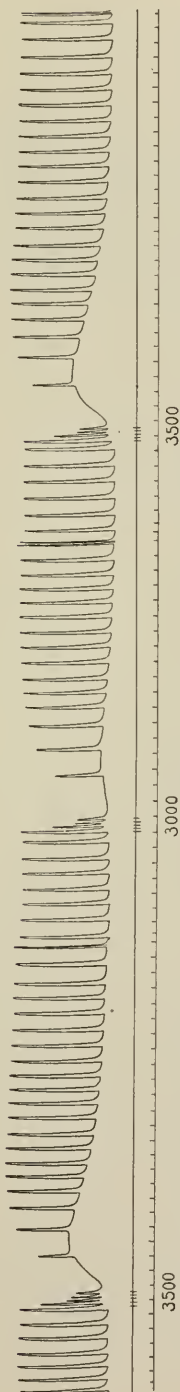


FIGURE 3. — Tonus curves from the auricle of the tortoise. The tonus contraction varies as the strength of the stimulus varies. The first tonus curve was obtained with the secondary coil at 3500 of the Kronecker scale, the second at 3000, and the third at 3500 again. The stimuli were the groups of break currents signalled in the middle line. The lowest line records every fifth second.

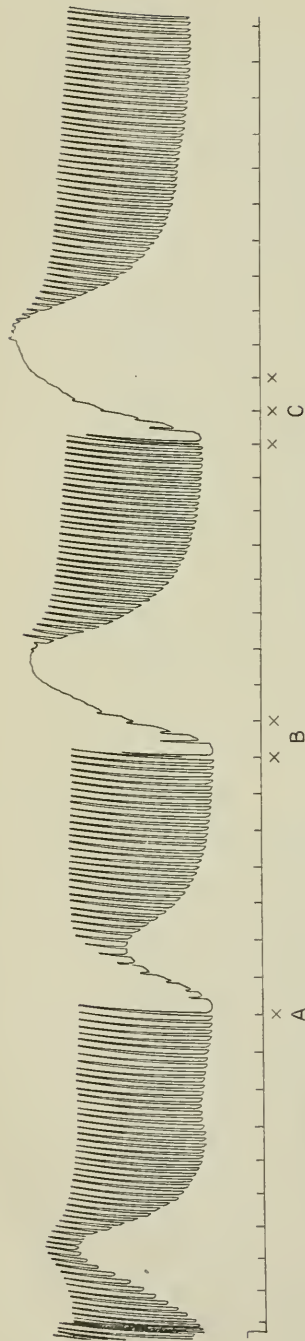


FIGURE 4.—Tonus curves from the auricle of the tortoise, showing the superposition (addition) of tonus contractions and the absence of a refractory period. The lower line records every fifteenth second. The first tonus contraction was spontaneous. At the points marked with crosses, the heart was stimulated with a break induction current, the secondary coil being at 5000 of the Kronecker scale.

spontaneous tonus contractions followed the last stimulus. In order to exclude any possible error from a spontaneous increase in tonus coincident with the electrical stimuli, the series of induction currents was now made 3500, 3000, 3500, 4000, 4500, 3500, 3500 units. The height of the tonus contractions rose and fell with the rise and fall in the intensity of the stimulus (Fig. 3). Finally, the secondary coil was placed at 3500 units and the number of the induction currents in the stimulus was diminished from 5 to 3, then to 2 and then to 1. The tonus contraction diminished *pari passu*.

It is well known that in skeletal muscle there is a considerable interval between the threshold value and the maximal contraction. In 1871 Dr. Bowditch discovered that in the heart muscle this interval does not exist or is too brief to be recorded. Exceptions to this law of "all or none" have from time to time been urged. The relation between the intensity of the stimulus and the height of tonus contraction here established may possibly explain these exceptions; they may have been tonus and not fundamental contractions. At all events, these cases should be studied again in the light of this new fact.

#### THE TONUS CONTRACTION HAS NO REFRACTORY PERIOD.

In the protocol of the experiment Nov. 26, 1902 (page 6), it was noted that the heart muscle is not refractory to induction currents in any phase of

tonus; in other words, additional tonus contractions can be produced by adequate stimuli, falling in any period of the preceding

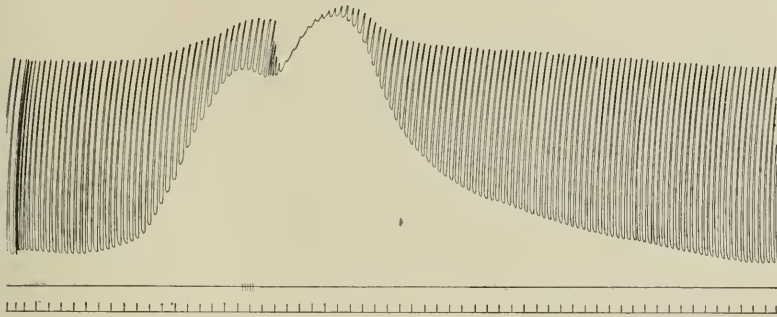


FIGURE 5.— Superposition of tonus contractions from the auricle of the tortoise. The first tonus contraction was spontaneous, the second from electrical stimulation. There is no refractory period. The stimulus was a series of break currents, the secondary coil being at 5000 of the Kronecker scale. The lowest line marks every fifth second.

tonus contraction. Proof of this is furnished in Figs. 4, 5, 6, and 7. In Fig. 4, *B*, it is clear that the second stimulus (a single break current) entered the muscle in the ascending phase of the

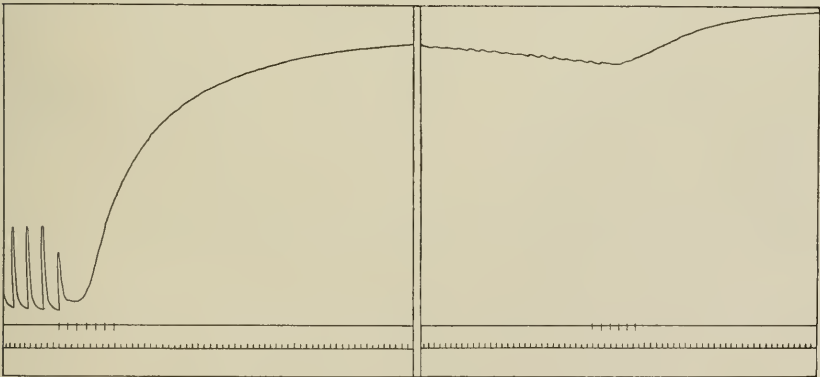


FIGURE 6.— From the auricle of the tortoise. The stimulus of seven break induction currents in nine seconds caused a tonus contraction lasting sixty-four minutes. One hundred and fifty seconds after the first group of stimuli, a second was applied. Addition was thereby secured. To save space, 100 seconds of the curve were omitted at the point marked by the vertical lines.

tonus contraction produced by the preceding stimulus, and it is equally clear that this second stimulus was effective, for the height of the tonus contraction at *B* produced by two stimuli is greater than

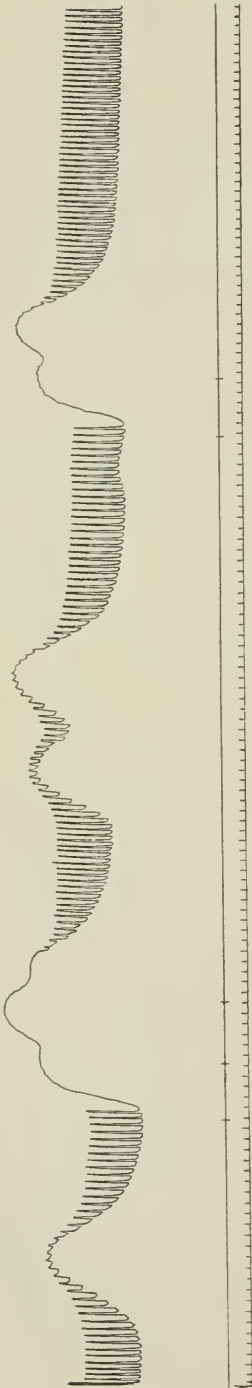


FIGURE 7. — Experiment, Dec. 1, 1902. Tonus curves from the anicle of the tortoise, showing that tonus contractions may be superposed, that tonus contractions are not refractory to stimuli, and that the latent period of tonus contractions does not vary with an increase in the tonus preceding the stimulus. The moments of stimulation are recorded in the middle curve and the time in five second intervals in the lowest curve.

that of the contraction at *A*, produced by only one stimulus. At *C*, in this same figure, three successive stimuli were applied, each falling in the ascending phase of the preceding tonus contraction, and each of the three produced an additional shortening. In Fig. 5, the second stimulation became effective almost at the crest of the preceding contraction. Fig. 6 is a record from a heart in which the tonus contractility or irritability was greatly increased. A brief stimulus caused very prolonged tonus contraction. Two and one half minutes after the beginning of the tonus contraction, and while it was still extreme the stimulus was repeated, and a further shortening was obtained. Other instances are shown in Fig. 7.

The absence of a refractory period suggests the addition of tonus contractions to form a true tetanus.

#### THE ADDITION OF TONUS CONTRACTIONS TO FORM A TRUE TETANUS.

Conclusive evidence of the addition of single contractions to form tetanus is furnished by Figs. 4, 5, 6, 7, and 8. In Fig. 4, at *B*, two stimuli and at *C* three stimuli, are summed, the second and third stimuli falling in the shortening due to the preceding stimulus. Traces of the fundamental contractions are seen upon the tonus curve. Fig. 5 presents a beautiful example of the addition of tonus contractions, and demonstrates clearly that the fundamental contractions do not enter into this addition. Fig. 6 is very instructive. It has already been stated that the tonus irritability in this heart was much exaggerated. A brief series of induction currents caused the pro-

longed tonus shown at the left of the figure. The fundamental contractions were at first invisible, but returned partially as the level of the curve fell slightly. After the extreme tonus contraction had lasted two and one half minutes the muscle was stimulated a second time and a clear addition secured. The stimulus was repeated twice at intervals of about one minute, and with the same result. The tonus contraction lasted sixty-four minutes. As the tonus fell the fundamental contractions returned, reaching their original size when the tonus had resumed its former level. In Fig. 7 the fundamental con-

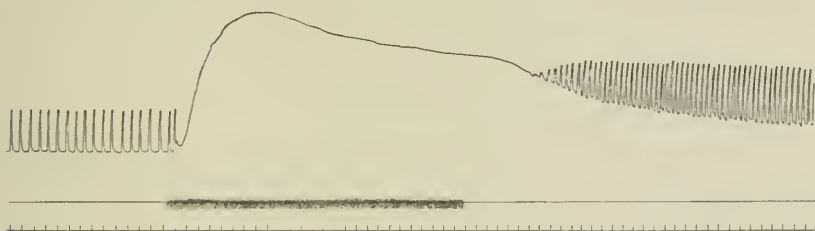


FIGURE 8. — Contractions of the auricle of the tortoise, showing tetanus of tonus in response to rapidly repeated break induction currents. Secondary coil at 5000 of Kronecker scale. The lowest line marks every fifth second.

tractions are almost invisible during the addition of tonus, yet the curves present such characteristic marks that their origin cannot possibly be in doubt. Fig. 8 records a tetanus of tonus secured by rapidly repeated induction currents. The resemblance between this curve and the tetanus curve of skeletal muscle will not escape notice.

Strictly speaking, the tetanus of tonus may be said to have no literature, for the present demonstration of the actual occurrence of this tetanus is the first that has been made. It is true that many observers have described a tetanus of the heart, while others have vigorously combated this idea. But this controversy has not concerned a tetanus of tonus, but a tetanus of fundamental contractions. One school has contended that the fundamental contractions could be fused in tetanus as the quick contractions of skeletal muscles are fused according to the hypothesis of Helmholtz. The opposing school has denied this fusion. No one has pointed out that the tetanus, which does indeed take place, is not a tetanus or fusion of the fundamental contractions, but a tetanus of the tonus contractions. It is my desire to discuss these earlier views in full in a communication on the superposition of tonus contractions, which will be the second paper of this series.



For the present it will be enough to give an example of the misunderstanding that has ascribed to the fundamental contractions the fusion that really belongs to the tonus contractions. For this purpose let us take the most recent study of this subject. On page 602 of his valuable paper on tetanic contraction of the mammalian heart, Professor Danilewsky writes as follows: "With the conditions of stimulation just mentioned, it is not difficult to convince one's self that the ventricle of the rabbit's heart is able to increase the mean heights of the systoles very markedly when the frequency of beat is great, whereby the apices of the systoles grow constantly higher than the normal and the diastolic dilatation becomes constantly smaller (superposition); these contractions and relaxations finally fuse in a straight line almost without serrations (tetanus)."<sup>1</sup>

It is evident that Professor Danilewsky speaks here of the fusion of fundamental contractions, whereas the tetanus observed by him was in reality a tetanus of tonus with partial or complete masking of the fundamental contractions.

Throughout this paper I have used the words "tetanus of tonus" with much reluctance, although they convey exactly the addition of tonus contractions demonstrated in this communication. Unfortunately, "tetanus of tonus" has been used by other writers in a different and less exact sense. The expression was introduced probably by Ranvier. In his "Leçons d'anatomie générale" Ranvier<sup>2</sup> presents Figs. 16 and 17, pp. 63 and 64, in illustration of what he calls tetanus of tonus. Fig. 16 is from the apex of the frog's heart, Fig. 17 from a red muscle

<sup>1</sup> DANILEWSKY: Archiv für die gesammte Physiologie, 1905, cix, p. 602. "Bei den erwähnten Reizungsbedingungen ist es nicht schwer, sich zu überzeugen, dass das Kaninchenherz, und zwar die Ventrikel, bei starker Frequenz der Systolen die mittlere systolische Höhe in ausgesprochener Weise zu steigern befähigt sind, wobei die Gipfel der Systolen immer höher werden als die Norm, und die diastolische Erweiterung immer kleiner wird (Superposition); diese Contractionen und Erschlaffungen verschmelzen endlich zu einer geraden Linie fast ohne irgend welche Zacken (Tetanus)."

<sup>2</sup> L. RANVIER: Leçons d'anatomie générale (1877-1878), Paris, 1880, pp. 63-64. "Mais, si nous employons un courant très fort, la décontraction du cœur, qui se fait lentement, n'a pas le temps de se compléter avant le début de la contraction suivante. Alors nous voyons peu à peu et progressivement s'élever sur le cylindre enregistreur le point de départ des systoles, tandis que celles-ci diminuent d'amplitude. Si l'expérience dure assez longtemps, la décontraction ne se fait plus du tout, et l'amplitude des pulsations se réduit à zéro (Fig. 16). Nous obtenons ainsi un tétanos dont la durée se prolonge longtemps encore après la cessation de la cause excitante."

(rabbit), each stimulated every six seconds by a strong induction current. In the legend of each figure he states that as the interval between two excitations is too short for complete relaxation, the heart remains contracted in tonus.<sup>1</sup> This idea is expanded in the text, as follows. "If a very strong current is employed, the relaxation of the heart, which takes place slowly, has not time to complete itself before the beginning of the following contraction. Then we see little by little the point of departure of the systoles rise progressively on the registering cylinder, while the systoles themselves diminish in amplitude. If the experiment continues long enough, the relaxation will not take place at all, and the amplitude of the pulsations will be reduced to zero. We have thus a tetanus which may last a long time after the exciting cause has ceased."

It seems clear that Ranvier did not have in mind a tetanus produced by the addition of successive tonus contractions. Ranvier's idea is one expression of the doctrine of "internal support" advocated in various forms by Von Frey, Grützner, Bottazzi, and others.

It has been shown in the present paper that addition is a property of the tonus contractions. To prevent confusion the word "tetanus" should be limited to such addition.

Helmholtz discovered the superposition, or, as it is better called, the addition of muscular contractions in skeletal muscle. The ideas of Helmholtz regarding the production of tetanus have become almost a dogma, especially in the teaching of physiology. It should be remembered that they include an hypothesis as well as a fact. The addition of single contractions in tetanus is a demonstrable fact. The production of tetanus by the addition of the fundamental contractions rather than by the addition of the tonus contractions is an hypothesis, the probability of which is greatly impaired by the observations here reported and by the growing conviction that there is no absolute difference between skeletal, cardiac, and smooth muscle. Within a few years we have gained indisputable evidence that all classes of muscle have both fundamental and tonus contractions. The tonus curves from human voluntary muscle and the gastrocnemius of the cat, secured in this laboratory,<sup>2</sup> are as

<sup>1</sup> La décontraction n'ayant pas le temps de s'opérer entre deux excitations, le cœur reste contracté en ton (tétanos de tonicité).

<sup>2</sup> T. A. STOREY: This journal, 1904-1905, xii, pp. 75-84. In the course of experiments at Stanford University on the irritability of human voluntary muscle, in 1902, Dr. STOREY observed rhythmic variations in the contraction of the

marked as those from the auricle of the tortoise and the intestine of warm-blooded animals. Appropriate stimulation will call forth tonus contractions in any muscle.

The time is ripe for greater simplicity and unity in our conception of the several varieties of muscle. Much would be gained by the following attractive and, as I hope to show in the next paper of this series, not improbable generalization:

Tetanus is essentially the same in smooth, cardiac, and skeletal muscle. In each it consists of fused tonus contractions upon which are placed the fundamental contractions. In some cases the fundamental contractions are visible, as in Figs. 4 and 5 of the present paper, in Fig. 43a in the recent article by R. Magnus,<sup>1</sup> and in many incomplete tetani of skeletal muscle, while in others the fundamental contractions are not visible to the eye though often demonstrable by special means. According to this hypothesis, the sustained shortening in tetanus is not produced by the fusion of fundamental contractions (Helmholtz's fusion), but by the fusion of tonus contractions.

abductor indicis muscle. On becoming a student in the Harvard Medical School, Dr. STOREY consulted me with regard to this observation, and at my suggestion did the work reported in his paper on tonus rhythms. Some of the experiments were performed on myself and other persons in the Harvard Laboratory, and the resulting curves confirmed the original observation. But I was not yet convinced that these extraordinary tonus curves were not of central origin. This doubt was finally laid at rest by obtaining similar tonus changes from the gastrocnemius muscle of a cat in which all connection with the central nervous system was cut off by section of the sciatic nerve. I mention these particulars here because the importance of the observations warrants a statement as to the critical spirit in which they were made.

<sup>1</sup> R. MAGNUS: *Archiv für die gesammte Physiologie*, 1905, cviii, pp. 1-71.

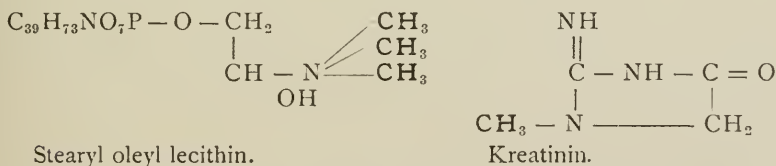


## RELATION OF KREATININ EXCRETION TO VARIATIONS IN DIET.

BY WALDEMAR KOCH.

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FOLIN<sup>1</sup> has recently pointed out the remarkable constancy with which kreatinin is excreted in man under widely varying conditions of diet. At the meeting of the American Physiological Society in 1904, Mendel<sup>2</sup> in a preliminary report gave some tables which indicate that the amount of kreatinin excreted bears a possible relation to the quantity of proteid metabolized. As his comparisons are for different individuals, however, they are not altogether conclusive, because Folin has pointed out that variations among individuals may be due to differences of weight and muscular development. At the same meeting I<sup>3</sup> presented a preliminary report of an investigation which indicated that lecithin might have some influence on kreatinin excretion. As a rich proteid diet usually means also a diet rich in lecithin, my results were rather in harmony with those of Mendel. Neither Mendel's nor my results afford any explanation, however, of the constant excretion observed by Folin under two very different dietaries, one containing about 15 gm. of lecithin and 16.8 gm. of proteid nitrogen, and the other less than 1 gm. of lecithin and 3.6 gm. of proteid nitrogen. On account of their chemical relationship, there is some basis for supposing that lecithin can take a part in the formation of kreatinin, as will be apparent from a consideration of the following formulæ :



<sup>1</sup> FOLIN: This journal, 1905, xiii, pp. 84, 118.

<sup>2</sup> MENDEL: This journal, 1904, xiii, p. xix.

<sup>3</sup> KOCH: This journal, 1904, xiii, p. xix.

Kreatinin, so far as we know, is the only constituent<sup>1</sup> of the urine which contains a methyl group attached to nitrogen, while lecithin and the closely related kephalin are, with the exception of small amounts of caffeine in tea and coffee, the only articles of diet which contain such groups attached to nitrogen. There is, moreover, some experimental evidence to show that methyl groups are transferred as a result of reactions going on in almost every cell of the animal body. Thus caffeine<sup>2</sup> is changed to dimethyl xanthin in man and to monomethyl xanthin in the dog.

His<sup>3</sup> found that pyridin takes up a methyl group on its passage through the body and is excreted as methyl pyridin. Finally, Hofmeister<sup>4</sup> studied carefully the formation of methyl telluride from tellurium in different organs. His results indicate that this transfer of methyl groups takes place to some extent in almost every cell and to a considerable extent in certain specialized cells. Hofmeister did not consider the attachment of this methyl group to nitrogen to be of any special significance, and consequently did not mention lecithin as a possible source, although he did consider possible the formation of cholin and kreatinin as a result of this methyl metabolism. On account of the promptness with which the transfer to tellurium takes place in surviving tissues without any apparent need of oxygen, he does not look upon the methyl groups as a residue derived from the oxidation of a straight chain hydrocarbon. If this methyl metabolism observed by Hofmeister is instrumental in the formation of kreatinin, the suggestion recently made by Seeman<sup>5</sup> with regard to the formation of kreatinin is without foundation.

Having been interested for some time in the food value<sup>6</sup> and in the intermediary metabolism<sup>7</sup> of lecithin, the above discussed relation of this lecithin to the methyl groups of kreatinin suggested to me a means by which the amount of lecithin and kephalin metabolized

<sup>1</sup> Methyl mercaptan contains methyl attached to sulphur, but need not be considered here, as it is not always present.

<sup>2</sup> ALBANESE: *Archiv für experimentelle Pharmakologie und Pathologie*, 1895, xxxv, p. 449.

<sup>3</sup> HIS: *Archiv für experimentelle Pharmakologie und Pathologie*, 1887, xxii, p. 253.

<sup>4</sup> HOFMEISTER: *Archiv für experimentelle Pharmakologie und Pathologie*, 1894, xxxiii, p. 198.

<sup>5</sup> SEEMAN: *Zeitschrift für physiologische Chemie*, 1905, xliv, p. 259.

<sup>6</sup> KOCH: *St. Louis Courier of Medicine*, 1905, xxxii, p. 344.

<sup>7</sup> KOCH: *Zeitschrift für physiologische Chemie*, 1902, xxxvii, p. 181.

might be measured. Thus, one molecule of lecithin would supply methyl groups for three molecules of kreatinin, or, approximately, 8 gm. of lecithin would yield 3.39 gm. of kreatinin. Likewise, one molecule of kephalin would supply its one methyl group to one molecule of kreatinin, or 8 gm. of kephalin would yield approximately 1.13 gm. kreatinin. In such a calculation a correction would have to be made for the exogenous kreatin introduced with the meat of the daily food. The possibility must also be considered that the body may have other means of removing methyl groups, as, for instance, oxidation to formic acid, a substance usually found in the urine.

With regard to the nitrogen of kreatinin, recent suggestions by Kutscher,<sup>1</sup> Czernecki,<sup>2</sup> and Seeman<sup>3</sup> point mainly to arginin or the closely related guanidin as the most probable source. Histidin does not contain enough nitrogen atoms in the proper relation in its molecule, and the other splitting products of the proteids lack the proper arrangement of carbon and nitrogen. It is interesting to note in this connection, therefore, that our daily diet contains more than enough arginin to account for all the kreatinin excreted. The excess of arginin is no doubt directly converted into urea in the intestine by Kossel's<sup>4</sup> arginase.

In order to determine if the amount of kreatinin excreted stands in some relation to the amount of lecithin and kephalin taken in with the food according to the calculation given above, the following experiment was undertaken. For the above determination of the kreatinin the very excellent method of Folin<sup>5</sup> could not be accurately followed, as the rather expensive Duboscq colorimeter was not available. Professor Mendel was, however, kind enough to request Mr. Clossen of his laboratory to compare standards with me and make some actual determinations. The results of his and of my readings will be given in a subsequent table. My determinations were made exactly as directed by Folin, except that the color comparisons were made in two graduated cylinders of even bore. The diameter of the cylinder was 1.67 mm. The height of column taken as standard was 45 mm. A column of  $\frac{2}{3}$  solution of potassium bichromate 45 mm. high corresponds in intensity of color to an equal

<sup>1</sup> KUTSCHER: *Zeitschrift für physiologische Chemie*, 1904, xliii, p. 108.

<sup>2</sup> CZERNECKI: *Zeitschrift für physiologische Chemie*, 1905, xliv, p. 295.

<sup>3</sup> SEEMAN: *Zeitschrift für physiologische Chemie*, 1905, xxxii, p. 344.

<sup>4</sup> KOSSEL and DAKIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 321.

<sup>5</sup> FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

column given by 7.87 mg. of kreatinin<sup>1</sup> treated according to Folin's method and made up to 500 c.c. In a private communication, Dr. Folin kindly suggested that for columns of liquid above 8 mm. it might be advisable for comparison to use a more dilute bichromate solution. I found, however, that on dilution the bichromate solution loses its characteristic red tinge which so admirably matches the color given by kreatinin with picric acid and sodium hydrate. No readings were taken with columns of less than 32 mm. or more than 67 mm.

The following table shows the results obtained in a metabolism experiment made upon three students. All the food taken by them was weighed, and all the urine excreted was collected.

Student.	Body wt.	Height.	Urea in 24 hrs.	Kreatin and kreatinin in 24 hours. Mr. Clossen's determination.	Kreatin and kreatinin in 24 hours. My determination.	Kreatin and kreatinin per kilo per 24 hours.	Remarks.
A	kilos 74.8	5 ft. 7 in.	grams 27.0	2.30	2.31	mgm. 30	Very muscular and active.
B	77.1	5 ft. 8 in.	20.0	2.07	2.16	27	Well developed, but less active.
C	68.0	5 ft. 10 in.	20.5	1.81	1.99	26	More active, but not so well developed.

It will be noticed that Mr. Clossen's results and mine agree as well as could be expected. Also that the amount of kreatinin per kilo body weight is a little higher than that found by Folin.

The amounts of kreatinin calculated and found agree as well as could be expected, considering that the above was merely intended for a preliminary calculation. As a matter of fact, the agreement may be merely a coincidence. If kreatinin is really a result of methyl metabolism, it might be possible to reduce its amount by causing the methyl to combine with some other substance, such as pyridin. One

<sup>1</sup> The sample of kreatinin (Merck) was free from kreatin and gave nitrogen  
37.3 : 36.9  
Calculated 37.1  
Moisture 2.0 %

Mr. CLOSSEN found, however, that 11.46 mg. kreatinin gave a color, 8.1 mm. of which corresponded to 8 mm. of  $\frac{1}{2}$  bichromate solution. According to FOLIN 10 mg. should give this result. The analysis would indicate that my sample was pure. I have therefore used the value found by Mr. CLOSSEN for my calculations.

gram of pyridin per day taken for a week gave, if anything, an increase in kreatinin, probably by stimulating methyl metabolism.

DAILY DIETARY AND KREATININ EXCRETION OF A, B, AND C.

Article of food.		Percentage of		Quantity in food of			Kreatinin.	
Moist.	Wt.	Leci- thin.	Keph- alin.	Leci- thin.	Keph- alin.	Kre- atin.	Calc. from methyl groups in food.	Found.
February 7, 1905.	grams			grams	grams	grams	grams	grams
Meat . . . . .	381	0.45 <sup>1</sup>	1.1 <sup>1</sup>	1.7	4.2	0.8 <sup>4</sup>	2.11	
Bread and pastry	1942	0.12 <sup>1</sup>	0.14 <sup>1</sup>	2.3	2.7	..	1.35	
Potatoes . . . . .	345	0.1 <sup>2</sup>	..	0.4	..	..	0.17	
Peas . . . . .	257	1.1 <sup>3</sup>	..	2.8	..	..	1.21	
Milk . . . . .	1166	0.043 <sup>1</sup>	0.042 <sup>1</sup>	0.5	0.5	..	0.30	
Fruit . . . . .	622	0.1 <sup>2</sup>	..	0.6	..	..	0.25	
Total . . . . .							5.39	6.18
<p><sup>1</sup> From analytical figures obtained in this laboratory by methods to be published shortly.</p> <p><sup>2</sup> Estimated, as no analyses are available.</p> <p><sup>3</sup> SCHULZE, C., and FRANKFURT, S.: <i>Die landwirtschaftlichen Versuchsstationen</i>, 1894, xliii, p. 315.</p> <p><sup>4</sup> Probable amount estimated.</p>								

In order to study variations of diet and better to control conditions the subsequent experiments were made on a dog. Experiment: A dog weighing 10 kilos was placed in a metabolism cage so arranged that all the urine could be obtained without contamination with fæces. The urine was not collected by catheter, but allowed to drain into a vessel beneath the cage. As the experiment was continued for a long time the loss by evaporation would merely introduce a slight constant error. The dog was only allowed to leave the cage occasionally between changes of diet, and care was taken not to lose any urine. Although at first uncomfortable, the animal soon became accustomed to the cage and gained weight during the experiment. The urine excretion under these conditions was found to undergo wide variations, as long as thirty hours sometimes elapsing before any urine was voided. As Dr. Mendel was kind enough to point out



to me that kreatinin is rapidly destroyed in an alkaline urine, special attention had to be paid to this point. In fact, some of my early observations were vitiated on this account. Particularly may be mentioned an attempt to feed the animal on an exclusive diet of milk and eggs, which gave rise to very liquid stools whereby the urine became contaminated, consequently leading to extremely low results for kreatinin. The addition of bread to the diet, however, obviated this difficulty, and tests made on the urine twenty-four and forty-eight hours after collecting showed no change. After that time, however, no reliance can be placed in the results.

The diets administered were essentially of two kinds, the one rich in lecithin and the other poor in lecithin. The rich lecithin diet consisted in the one case of bread, milk, and eggs, in the other case, of bread, milk, and lecithin (made from eggs by the Actien Gesellschaft für Anilin Fabrikation and sold under the trade-mark A. G. F. A. Lecithin: by analysis two-thirds kephalin). The low lecithin diet consisted of bread and milk. It would have been preferable to have given a diet entirely free from lecithin, but this is almost impossible. Milk freed from all its fat by the cream separator still retains the larger part of its lecithin. The preparation of sufficient proteid free from lecithin for an experiment which needed to be continued for the length of time above stated would involve much difficulty. Moreover, there is always the danger of making the diet unpalatable. In fact, a dog that will live for months on bread and milk alone, as the subject of this experiment did, I should say is rather uncommon.

The complete observations are given in the tables at the end of the paper. The following table gives the results in a condensed form. On account of the extreme daily variations (see tables at end of paper) the maximum, minimum, and mean excretions for each period are given, as well as the average. The agreement between the mean and the average is as close as can be expected.

The Roman numerals indicate the order in which the diets followed one another. The second low lecithin final period (V) followed a ten-day period of feeding on white of egg (IV not given), which later was found to contain a little lecithin, and during which the average kreatinin excretion was increased a corresponding amount to 0.247 gm. per day. Diet VII followed diet VI, which contained the A. G. F. A. Lecithin. No final period of low lecithin feeding could be carried out after this, as the experiment had to be inter-

rupted. No kreatin was ever found in the urine during the above experiments.

The above results again emphasize the remarkable constancy of kreatinin excretion, especially shown in the two final periods of low

DIETARY RICH IN LECITHIN AND KEPHALIN.								
No. of table.	Days of exp.	Dietary. 24 hours.	Lecithin and kephalin in dietary.	Kreatinin calc. from lecithin and kephalin.	Kreatinin found. Av. in 24 hrs.	Kreatinin found. Mean in 24 hrs.	Kreatinin found. Max. in 24 hrs.	Kreatinin found. Min. in 24 hrs.
I	15	grams Milk, 350-400 Bread, 125 Eggs, 90	grams l. 1.5 k. 3.0	grams 1.05	grams <b>0.260</b>	grams 0.270	0.394	0.146
VI	18	Milk, 450 Bread, 200 A. G. F. A. Lecithin, 1.5	l. 1.0 k. 1.5	0.63	<b>0.267</b>	0.269	0.354	0.184
DIETARY LOW IN LECITHIN AND KEPHALIN (AFTER PERIOD).								
II	11	Milk, 400 Bread, 200	l. 0.43 k. 0.47	0.246	<b>0.261</b>	0.273	0.363	0.183
VII	11	Milk, 450 Bread, 200	l. 0.43 k. 0.47	0.246	<b>0.253</b>	0.240	0.324	0.156
DIETARY LOW IN LECITHIN AND KEPHALIN (FINAL PERIOD).								
III	22	Milk, 450 Bread, 200	l. 0.43 k. 0.47	0.246	<b>0.234</b>	0.219	0.295	0.142
V	26	Milk, 450 Bread, 200	l. 0.43 k. 0.47	0.246	<b>0.235</b>	0.233	0.334	0.132

lecithin diet, the averages of which agree almost exactly in spite of very large daily variations. The mean values also agree quite closely. The kreatinin excretion per kilo comes remarkably near that observed for man (24-26 mg.). The calculated and observed kreatinin agree very well in the case of III and V (difference 5 per cent), in fact much better than in the preliminary calculation previously considered. With an increased amount of lecithin in the dietary, however, there is only a very slight corresponding increase in the amount of kreatinin excreted. This does not prove that the methyl

groups of lecithin have no relation to the methyl groups of kreatinin, any more than the fact observed by Folin, that a large increase in proteid is not followed by an increase in kreatinin, proves that the nitrogen of kreatinin is not derived from proteid nitrogen. It merely indicates that the metabolism which gives rise to kreatinin is not easily influenced by changes in diet, but is probably under physiological control. The observations of Gregor<sup>1</sup> on muscular exercise and kreatinin excretion, confirmed by myself, emphasize this point.<sup>2</sup> The above results, however, give some clue concerning the fate of this excess of lecithin, as observations by Rubow,<sup>3</sup> also confirmed by myself but not yet published, indicate that muscle has the power of storing lecithin. The persistence of a high kreatinin excretion in II and VII, after the lecithin in the diet has been reduced to a lower point than can account for all the kreatinin excreted, indicates that the stored lecithin is being drawn upon. In Diet II following I, in which the greater excess of lecithin has been given, the excretion in the after period is higher than in VII following VI, where the excess was not so great. That the larger amounts of lecithin given in I and VI were really absorbed from the intestine was demonstrated by an occasional examination of the fæces, which never contained any lecithin. After Diet I, which contained such a large amount of lecithin, it took two months before the final low result of Diet III was obtained. These observations explain why Folin obtained such a constant kreatinin excretion for twelve to eighteen days after a period of feeding on from ten to twelve eggs a day. There is, of course, the possibility already mentioned, that the body has in addition another method of taking care of the excess of methyl groups; namely, oxidation to formic acid (which possibility was not investigated). It would be interesting to note if, under a lecithin free diet long continued, the amount of kreatinin could finally be reduced, or if in its stead glyco-cyamidine (which only differs in lacking the methyl group) would be excreted. The methods employed by Czernecki in his investigation of this point were unfortunately so poor that no conclusions can be drawn from his results.

It is quite possible that the methyl group gives to kreatinin its relative stability and prevents its breaking up into urea, as more

<sup>1</sup> GREGOR: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 98.

<sup>2</sup> See also ALSBERG and FOLIN: This journal, 1905, xiv, p. 72.

<sup>3</sup> RUBOW: Archiv für experimentelle Pharmakologie und Pathologie, 1905, lii, p. 173.



recent investigations do not support the older supposition that kreatinin is a source of urea. The attachment of the methyl group to the purin ring is evidently not so firm as in the case of kreatinin, otherwise we might expect some of the purin bases of the urine to play a rôle in the removal of this group from the body.

#### SUMMARY.

Kreatinin is excreted with remarkable constancy by the dog as well as by man. The extreme daily variations in the case of the dog do not affect the final average. The excretion per kilo body weight for twenty-four hours is very nearly the same for both (24-26 mg. for the dog; 26-30 mg. for man).

Under ordinary conditions of diet, the methyl groups of the lecithin and kephalin ingested can all be accounted for by the methyl groups of the kreatinin excreted. With an excess of lecithin and kephalin this is not the case, although the kreatinin is undeniably increased. This increase is due to the lecithin and kephalin of the egg, and not to some other constituent.

Kreatinin is probably a better index of methyl metabolism than of the lecithin and kephalin metabolized, although under ordinary conditions the two seem closely related.

Further experiments will have to determine if physiological activity is capable of influencing this metabolism to a greater extent than the presence of an excess of methyl groups in the form of lecithin and kephalin.

If kreatinin bears the relation to methyl metabolism suggested in the above paper, it should be possible to demonstrate the presence of kreatin in every tissue having such a metabolism. The presence of kreatin in striated muscle is a well-known fact. I have also found it in the heart muscle and in the testicle, and am continuing the investigation of other tissues.

In conclusion, it gives me great pleasure to thank Dr. Mendel and Mr. O. E. Clossen for their kind co-operation in this work.

## I. DIET RICH IN LECITHIN AND KEPHALIN.

DIETARY: MILK, 350-400 C.C.; BREAD, 125 GM.; EGGS, 90 GM.

Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column. Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1904 Nov. 15	235	10	34	45	0.245
" 16	} 385	10	32	45	} 0.213
" 17					
" 18	325	10	38	45	0.303
" 19	410	10	38	45	0.383
" 20	} 355	10	43	45	} 0.146
" 21					
" 22	} 375	5	50	45	} 0.265
" 23					
" 24	320	10	45	50	0.280
" 25	400	10	52	45	0.272
" 26	310	10	54	45	0.204
" 27	425	10	54	45	0.279
" 28	280	10	45	58	0.284
" 29	300	5	54	45	0.394
Total . . .	+120 c.c. in 15 days.			Average . . . 0.260	

II. DIET LOW IN LECITHIN AND KEPHALIN (AFTER PERIOD).

DIETARY: MILK, 400 C.C ; BREAD, 200 GM.					
Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1904 Dec. 1	300	10	58	45	0.183
" 2	475	20	32	45	0.266
" 3	} 534	10	45	54	} 0.252
" 4					
" 5	415	10	45	50	0.363
" 6	} 415	5	54	45	} 0.272
" 7					
" 8	250	10	45	56	0.245
" 9	} 490	10	45	50	} 0.214
" 10					
" 11	345	10	45	54	0.326
Total . . . 3224 c.c. in 11 days.			Average . . . 0.261		

## III. DIET LOW IN LECITHIN AND KEPHALIN (FINAL PERIOD).

DIETARY: MILK, 450 c.c.; BREAD, 200 GM.					
Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column. Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1905 Jan. 27	385	10	58	45	0.235
" 28	330	10	50	45	0.234
" 29	} 1100	20	45	54	} 0.260
" 30					
" 31	285	10	45	52	0.260
Feb. 1	260	10	45	45	0.204
" 2	310	10	45	45	0.244
" 3	400	10	63	45	0.225
" 4	540	20	45	45	0.212
" 5	} 725	10	60	45	} 0.214
" 6					
" 7	190	10	45	65	0.216
" 8	610	20	45	50	0.267
" 9	450	10	54	45	0.295
" 10	180	10	45	45	0.142
" 11	300	10	56	45	0.189
" 12	} 975	20	45	67	} 0.285
" 13					
" 14	215	10	45	67	0.252
" 15	500	20	45	56	0.245
" 16	250	15	45	54	0.157
" 17	600	20	45	50	0.262
Total . . .	8605 c.c. in 22 days.			Average . . .	0.234

V. DIET LOW IN LECITHIN AND KEPHALIN (FINAL PERIOD).

DIETARY: MILK, 450 C.C.; BREAD, 200 GM.					
Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column. Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1905 March 5	} 710	10	56	45	0.224
" 6					0.225
" 7	} 475	10	45	67	0.278
" 8					0.279
" 9	350	10	50	45	0.248
" 10	280	10	58	45	0.171
" 11	360	15	41	45	0.207
" 12	} 1150	10	61	45	0.334
" 13					0.334
" 14	} 300	10	45	50	0.132
" 15					0.132
" 16	510	15	45	50	0.297
" 17	350	15	45	63	0.256
" 18	515	20	50	45	0.182
" 19	} 720	10	50	45	0.254
" 20					0.254
" 21	300	10	45	58	0.304
" 22	360	15	45	54	0.226
" 23	360	15	45	54	0.226
" 24	310	15	45	45	0.162
" 25	390	15	45	54	0.246
" 26	700	15	58	45	0.285
" 27	360	15	50	45	0.170
" 28	} 525	10	45	54	0.248
" 29					0.249
" 30	330	10	63	45	0.186
Total . . . 9355 c.c. in 26 days.				Average . . . 0.235	

## VI. DIET RICH IN LECITHIN AND KEPHALIN.

DIETARY: MILK, 450 C.C.; BREAD, 200 GM.; LECITHIN (A. G. F. A.), 1.5 GM., AS AN EMULSION IN 50 C.C. WATER.					
Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column. Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1905 April 7	640	15	45	45	0.336
" 8	675	15	45	45	0.354
" 9	} 720	15	45	50	0.211
" 10					0.211
" 11	370	10	45	45	0.291
" 12	390	15	50	45	0.184
" 13	490	15	45	45	0.256
" 14	560	15	45	50	0.327
" 15	390	15	50	45	0.184
" 16	} 770	15	45	52	0.322
" 17					0.323
" 18	325	10	45	54	0.307
" 19	475	15	52	45	0.216
" 20	345	10	54	45	0.227
" 21	480	15	45	50	0.280
" 22	605	15	56	45	0.255
" 23	350	10	50	45	0.248
" 24	350	10	45	45	0.276
Total . . . 7935 c.c. in 18 days.			Average . . . 0.267		

VII. DIET LOW IN LECITHIN AND KEPHALIN (AFTER PERIOD).

DIETARY: MILK, 450 c.c. ; BREAD, 200 GM.					
Date.	Total urine.	Amount of urine used for determination.	Height of column. Kreatinin solution.	Height of column. Bichromate solution.	Kreatinin in 24 hours.
	c.c.	c.c.	mm.	mm.	grams
1905 April 25	250	10	45	56	0.245
" 26	460	15	45	50	0.269
" 27	590	15	45	47	0.324
" 28	} 650	10	56	45	0.205
" 29					0.206
" 30	} 660	15	50	45	0.156
May 1					0.156
" 2	} 420	5	50	45	0.297
" 3					0.297
" 4	455	10	52	45	0.310
" 5	520	15	45	52	0.316
Total . . . 4005 c.c. in 11 days.				Average . . . 0.253	

# ON RESISTANCE TO LACK OF OXYGEN AND ON A METHOD OF INCREASING THIS RESISTANCE.

By WALES H. PACKARD.

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## I. INTRODUCTION.

THE most generally accepted theory of oxidation has been up to the present time that of Hoppe-Seyler.<sup>1</sup> According to this theory processes of fermentation take place in the protoplasm which result in the formation of nascent hydrogen. This combines with atmospheric oxygen, if it is present, forming water and setting free atoms of oxygen ( $H_2 + O_2 = H_2O + O$ ). The nascent oxygen thus formed attacks the protoplasm, producing the oxidations characteristic of living matter at comparatively low temperatures. If atmospheric oxygen is not present the nascent hydrogen reduces substances in the cell and forms entirely different products, which are more or less poisonous. This theory did not account for anaërobic respiration in which the protoplasm carries on oxidations in the complete absence of free oxygen, or in the presence of only traces of it.

Mathews<sup>2</sup> has recently brought forward an hypothesis which differs somewhat from that of Hoppe-Seyler, and which is based on the recent work of Armstrong, Nef, and others. Armstrong<sup>3</sup> has shown that in ordinary oxidations the oxygen of the air does not combine directly with carbon to form carbon dioxide, or with hydrogen to form water. The presence of a certain amount of water is essential in all processes of oxidation, and the substances undergoing oxidation are first hydroxylized. The function of the atmospheric oxygen

<sup>1</sup> HOPPE-SEYLER: *Zeitschrift für physiologische Chemie*, 1877, i, p. 126.

<sup>2</sup> MATHEWS: *Biological Bulletin*, 1905, viii, p. 331.

<sup>3</sup> ARMSTRONG: *Chemical News*, 1904, xc, p. 25; *Transactions of the chemical society of London*, 1903, lxxxiii, p. 1088.



is simply to act as a depolarizer, to unite with the nascent hydrogen formed from the water. According to Mathews a somewhat similar process takes place in respiration. "Certain active particles in the protoplasm attack the water which is decomposed into oxygen and hydrogen. The oxygen combines with substances of the protoplasm, thus oxidizing them; the hydrogen is either set free in gaseous form, or it is united with atmospheric oxygen to form water; or it combines with other substances in the protoplasm." In other words, respiration is the dissociation of water with the liberation of hydrogen, and the real respiration is brought about not by the oxygen of the air but by that of water. If atmospheric oxygen is present, it unites with the hydrogen set free from the water, thus acting as a depolarizer. According to this theory aërobic and anaërobic respiration are identical; the only difference is that the anaërobic protoplasm is a powerful enough reducing agent to drive the hydrogen out of the water and let it escape as free hydrogen. The aërobic protoplasm, on the other hand, is less powerful, and requires the presence of oxygen to combine with the hydrogen. Barnes<sup>1</sup> has also reached very similar conclusions. Dr. Mathews further suggests that "as atmospheric oxygen thus acts the part only of a depolarizer, any other oxidizing agent, that is, any other substance which unites readily with nascent hydrogen, can replace the atmospheric oxygen and permit oxidation to go on in the absence of air." Lævulose is perhaps such a depolarizing substance. Maze<sup>2</sup> found that if lævulose were present alcohol was oxidized to acetic acid by a certain bacterium in the absence of air. The lævulose in this case united with the hydrogen formed, being itself changed to mannite. It was with a view of testing this point and others that at the suggestion of Dr. Mathews the following experiments were undertaken. The general problem was, How long will certain animals resist a lack of oxygen, and how may this resistance be increased.

## II. RESISTANCE TO LACK OF OXYGEN.

That animals show a very unequal resistance to lack of air was shown more than a century ago by Spallanzani, who experimented by introducing his organisms into hermetically sealed vessels. Further experiments have been made by Bunge, Newport, Weinland, and others.

<sup>1</sup> BARNES: *Botanical Gazette*, 1905, xxxix, p. 81.

<sup>2</sup> MAZE: *Annales de l'Institut Pasteur*, 1904, xviii, p. 277.

Bunge,<sup>1</sup> in his work upon the respiration of intestinal parasites and mud-dwelling organisms, showed that parasites in the intestine of warm-blooded animals must live practically in the absence of oxygen, as the contents of the intestine contain no free oxygen. Worms living in mud are also subject to similar conditions, decomposition processes, with the formation of reducing substances, keeping the oxygen absent. The following list showing the time that different animals will live in oxygen-free fluids is taken from Bunge's work :

Parasitic nematodes	4-6 days	Snails . . . .	½ day.
Leeches . . . .	4 days	Crayfish . . . .	few hours
Planarians . . . .	3 "	Water beetles . .	" "
Earth worms . . .	1 "	Mites . . . . .	" "

Weinland<sup>2</sup> found that *Ascarus lumbricoides* from the intestine of the hog would live in boiled 1 per cent sodium chloride solution from four to six days. Newport<sup>3</sup> states that butterfly larvæ will live in an atmosphere of hydrogen for twelve hours.

It seemed advisable to determine for some of the common animals at Woods Hole the length of time that they would live in sea-water which had been freed from oxygen. The following method was used in the experiments. The sea-water was boiled for an hour, the water lost in evaporation being replaced by boiled distilled water. For the experiment the previously boiled water was placed in a 250 c.c. flask and a stream of hydrogen passed through it. The hydrogen was generated in the ordinary manner in a Kipp apparatus, and was thoroughly washed by passing through potassium hydrate solution, potassium permanganate solution, and distilled water. In several test experiments a solution of hæmoglobin was introduced into the sea-water to determine whether or not the oxygen had been completely removed. It was found that the hydrogen must be passed through the water from one and a half to two hours before the oxy-hæmoglobin as shown by the spectroscope was completely reduced. The animals to be tested were introduced into the flask while a rapid stream of hydrogen was passing through, and the hydrogen was continually passing during the entire course of the experiment. Table I gives the results of the experiments.

<sup>1</sup> BUNGE: *Zeitschrift für physiologische Chemie*, 1883, viii, p. 48; 1888, xii, p. 565; 1889, xiv, p. 318.

<sup>2</sup> WEINLAND: *Zeitschrift für Biologie*, 1901, xlii, p. 348.

<sup>3</sup> NEWPORT: *Philosophical transactions*, 1836, cxxvi, p. 529.

Several experiments were made on each species, and in the table the minimum, maximum, and the average time of all the experiments are given. Considerable individual variation in each species will be noticed. This depends somewhat upon the temperature and the condition of the animal at the time of the experiment, and whether or not it had been kept in an aquarium for some time previous. No correction was made for temperature beyond repeating the experi-

TABLE I.  
LENGTH OF LIFE IN COMPLETE LACK OF OXYGEN.

	Minimum.	Maximum.	Average of all experiments.
	hrs. min.	hrs. min.	hrs. min.
Fundulus magalis . . . . .	40	1 05	51
Fundulus heteroclitis . . . . .	1 30	4 55	3 34
Ctenolabrus . . . . .	35	55	45
Apeletes (Stickleback) . . . . .	22	32	28
Gelasimus (Fiddler crab) . . . . .	12 00	24 30	18 11
Eupagurus (Hermit crab) . . . . .	11 30	15 00	14 15
Panopæus (Mud crab) . . . . .	22 00	26 15	24 35
Palæmonetes . . . . .	10	24	15
Talorchestia . . . . .	44	1 18	1 03
Nereis . . . . .	22 00	35 00	27 00
Amphitrite . . . . .	18 00	22 00	21 00

ment a number of times and taking the mean. In the later experiments only fresh material just brought into the laboratory was used. It will be noticed from the table that there is a marked difference in the resistance of two common fish — *Fundulus heteroclitis* and *Ctenolabrus*. Loeb<sup>1</sup> has reported a similar difference in the resistance of both the eggs and embryos of these animals. Loeb<sup>2</sup> also found that in *Fundulus* the embryo was more sensitive to lack of oxygen the older it was. The fertilized egg could continue its development after having lain in an oxygen vacuum for four days, while the young fish just hatched could resist less than forty-eight hours

<sup>1</sup> LOEB: Archiv für die gesammte Physiologie, 1895, lxii, p. 249.

<sup>2</sup> LOEB: *Ibid.*, 1894, lv, p. 530.

in the oxygen vacuum. This power of resistance evidently decreases with the growth of the animal, until in the adult stage it is three or four hours only.

The very high resistance of the worms and some of the mud-dwelling crustacea noted in Table I coincides with the observation of Bunge. On the other hand the hermit crab lives normally in the presence of abundant oxygen.

### III. EFFECT OF INCREASED ALKALINITY OF THE BLOOD ON RESISTANCE TO LACK OF OXYGEN.

It has been known for some time that an increase in the alkalinity of the protoplasm favored oxidation while a decrease caused it to cease. Zoethout<sup>1</sup> has shown that in paramœcium the resistance to lack of oxygen, or to poisons which prevent oxidation, may be increased by a very small percentage of alkali ( $\frac{1}{400} - \frac{1}{2000}$  per cent KOH). The result is attributed to the antagonistic effect which the alkali has upon the poisons produced in the protoplasm by lack of oxygen. It seemed advisable to extend these experiments to some of the higher animals to test the effect of alkali on their resistance to lack of oxygen, by increasing the alkalinity of the blood. It is evident from the very great individual variation in resistance to lack of oxygen in any one species (see Table I) that any effect of the injection of alkali can be demonstrated only by the use of large numbers of individuals. *Fundulus heteroclitis* was selected for experiment as being easily obtained in sufficient quantities. Sodium bicarbonate was the alkali chosen. As is well known, dilute solutions of the acid sodium carbonate react alkaline. This is due to the hydrolysis of the acid salt by water setting free some hydroxyl ions:  $\text{NaHCO}_3 + \text{H}_2\text{O} = \text{Na}^+ \text{OH}^- + \text{H}_2\text{CO}_3$ . The amount of hydrolysis is slight and the carbonates and bicarbonates are much less irritating to animal tissues than the stronger hydrates. In order to avoid osmotic effects with the blood the bicarbonate was used in the strength of a  $\frac{5}{16}$  m solution as being approximately isotonic with the blood of marine teleosts.<sup>2</sup> The method of the experiment was as follows: The fish were injected in the body cavity with three to eight drops (according to the size of the animal) of the sodium bicarbonate solution by means of a hypo-

<sup>1</sup> ZOETHOUT: This journal, 1899, ii, p. 220.

<sup>2</sup> GARREY: Biological bulletin, 1905, viii, p. 257.

dermic syringe. They were then left for some time that the alkali might be absorbed. This was evidently very quickly done, as the length of time seems to make little difference in the experiment. A number of fish (usually 10 injected and 10 controls) were placed in a litre flask which was then completely filled with ordinary sea-water and tightly stoppered for the exclusion of all air. The animals very quickly exhausted the supply of oxygen in the water and thus were under conditions of lack of oxygen. A comparison of the tables will show that animals lived under these conditions but slightly longer than when elaborate methods were used to remove the oxygen from the water. That we are dealing here with phenomena of lack of oxygen and not with poisoning effects of the accumulation of carbon dioxide has been demonstrated by Jolyet and Regnard.<sup>1</sup> According to these authors a litre of sea-water contains 7.9 c.c. O; 15 c.c. N; 23.8 c.c. CO<sub>2</sub> (3.8 c.c. free: 20 c.c. in combination). If oxygen is supplied to fish in a closed vessel they will live in water in which the amount of carbon dioxide has increased to 211 c.c. per litre. Under the conditions of the experiment this accumulation would not take place until long after the relatively small amount of oxygen in sea-water had been entirely exhausted. The fish were left in the closed flask from half an hour to an hour after all movements of the animals had ceased. They were then removed to fresh, running sea-water and left from three to four hours for reviving. The length of time the animals should be left in the flask could not always be accurately determined. It depends a great deal upon the condition of the animals and the temperature; but as the controls and injected animals were subjected to the same conditions, the absolute length of time does not enter into the experiment. If they were not left long enough all the animals would revive, and in a few cases they were left too long and all were dead. Table II shows the results of the experiments.

It will be seen from the summary that out of the 183 individuals alive at the end of the experiments 55 or 30 per cent were controls and 128 or 70 per cent were injected; while out of 197 dead, 135 or 69 per cent were controls and only 62 or 31 per cent were injected. The 30 per cent alive controls are easily accounted for if we consider the variation in individual resistance. In every lot of animals there are always a few whose individual resistance is so great that they

<sup>1</sup> JOLYET et REGNARD: Archives de physiologie, 1877, pp. 44 and 584.



TABLE II.  
 FUNDULUS INJECTED WITH  $\frac{5}{16}m$   $\text{NaHCO}_3$ .

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
61	hrs. min. 10	hrs. min. 2 30	2	3	5	0
62	15	2 40	2	4	4	2
65	20	2 50	3	6	8	1
66	10	2 40	3	3	5	1
67	4 40	2 20	1	4	3	2
69	20	2 10	2	8	4	6
70	30	2 05	3	5	2	6
75	4 25	2 15	3	5	5	3
76	3 40	2 20	0	5	3	2
77	3 15	2 25	2	8	4	6
79	29 15	3 25	6	4	9	1
80	44 30	4 40	3	7	5	5
81	30	3 25	0	10	8	2
84	16 40	4 15	4	6	7	3
85	7 20	4 00	2	8	6	4
86	40	4 30	5	2	7	0
87	40	4 15	2	8	10	0
88	35	4 40	2	8	4	6
96	16 40	4 40	0	5	1	4
103	40	3 20	3	7	8	2
113	35	2 30	3	12	10	5
114	25	2 15	4	7	10	1
Total . . . . .			55	135	128	62

## SUMMARY.

Alive, 183 . . . . Controls, 55 (30%)      Injected, 128 (70%)  
 Dead, 197 . . . . "      135 (69%)      "      62 (31%)

will remain alive for the length of time of the experiment. The same reason also accounts for the 31 per cent dead animals which were injected. These are the animals whose individual resistance is so slight that even with the increased resistance given by the alkali, they are killed in the length of time of the experiment. The effect of the alkali lasts at least from one to two days. Evidently the alkali is not removed from the system very quickly.

In order to avoid the objection that perhaps the injection of a large amount of fluid may render the animals more resistant to lack of oxygen, the following two experiments are given. The animals were

TABLE III.  
FUNDULUS INJECTED WITH DISTILLED WATER.

Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 2 00	hrs. min. 2 10	7	3	7	3
2 05	2 15	6	4	6	4

injected with five drops of distilled water. In each case an equal number of controls and injected died, showing that a mere increase of fluid had no effect either favorable or detrimental.

#### IV. EFFECT OF DECREASED ALKALINITY OF THE BLOOD ON RESISTANCE TO LACK OF OXYGEN.

Table IV gives the result of the experiments to determine the effect of the injection of acid on the resistance to lack of oxygen. The animals were injected with  $\frac{m}{250}$  or  $\frac{m}{100}$  solution of acetic acid.

Experiments with  $\frac{m}{500}$  acid showed that acid of that strength had no appreciable effect on the animals. A control experiment in which 50 animals were injected with  $\frac{m}{100}$  acetic acid showed that the animals would endure acid of that strength. The animals lived in the aquarium with only the few deaths that would normally occur. From the summary it will be seen that there is exactly the reverse effect of the injection of the alkali. Of the 88 alive animals 59 (67 per cent) were controls and 29 (33 per cent) were injected and of the 108 dead 39 (36 per cent) were controls and 69 (64 per cent)



were injected. The detrimental effect of the acid is not quite as marked as the favorable effect of the alkali.

A comparison of the average length of life after injection of acid with that after the injection of alkali is instructive. Taking ten experiments which were carried out under conditions as near alike as

TABLE IV.  
FUNDULUS INJECTED WITH  $\frac{m}{100} - \frac{m}{250}$  ACETIC ACID.

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
92	min. 10	hrs. min. 3 40	6	4	4	6
94	10	3 30	7	3	5	5
98	15	4 00	5	5	4	6
99	5	4 00	9	1	5	5
102	20	3 20	3	7	0	10
107	20	2 25	4	6	1	9
110	35	2 40	10	0	4	6
111	5	2 05	3	5	1	7
112	10	1 55	6	4	2	8
115	15	2 15	6	4	3	7
Total . . . . .			59	39	29	69

## SUMMARY.

Alive, 88 . . . . . Controls, 59 (67%)      Injected, 29 (33%)  
Dead, 108 . . . . . " 39 (36%)      " 69 (64%)

possible as regards temperature and freshness of the animals, we find that the average time in the flask for the animals injected with acid was two hours, fifty-nine minutes; for the animals injected with alkali, three hours, fifty-five minutes, an increase of fifty-six minutes in favor of the latter. It is possible that under the confined conditions in the flask the acid or alkali diffused into the water and affected the controls as well as those injected. Thus the length of life of the entire number in the flask with the acid would be shortened, while that of those with the alkali would be lengthened.

Rosenow<sup>1</sup> in some studies on pneumonia and pneumococcus infections reports the formation of acids by the growth of the pneumococcus in the consolidated lung and blood of pneumonic patients, and suggests that some of the symptoms of pneumonia are due to an acid intoxication. In the service of Dr. Frank Billings<sup>2</sup> at the Presbyterian Hospital, Chicago, sodium bicarbonate has been used clinically in the treatment of pneumonia for the past two years with favorable results. It is possible that these are conditions similar to those in our experiments. The effect of the acid in the blood is partly, at least, an interference with the processes of oxidation in the tissues which is relieved by the large doses of alkali with its favorable action on oxidation.

Loeb<sup>3</sup> has found that the addition of acids to sea-water delayed the development of the larvæ of sea-urchins, while the addition of alkalies accelerated the rate of development and growth. Loeb<sup>4</sup> also reports that the addition of alkalies to Van't Hoff's artificial sea-water favored the regeneration and growth of Tubularians. These facts may be interpreted as being the result of the favorable action of alkalies on processes of oxidation in the protoplasm.

Mathews<sup>5</sup> has given a possible explanation of the effect of acids and alkalies upon oxidation in terms of the solution-tension of the elements. "Respiration is carried on chiefly by the oxygen ions. When these are increased in number, as they are increased by making the protoplasm more alkaline, their solution-tension falls. They give up their negative charges so much the more rapidly and easily. When on the other hand we increase the acidity or reduce the alkalinity, there is a reduction of the hydroxyl ions, their solution-tension thereby increases, they no longer give up negative charges to protoplasm, and respiration is brought to a stop. Life and respiration of any cell is checked, as soon as the number of the hydroxyl ions is reduced so far that the solution-tension of the ion is greater than the solution-tension of the protoplasm." Whatever the explanation may be there is no doubt of the fact that many oxidations similar to those occurring in protoplasm are assisted by alkalies and retarded by acids.

<sup>1</sup> ROSENOW: Journal of infectious diseases. 1904, i, p. 280: Journal of the American Medical Association, 1905, xlv, p. 871.

<sup>2</sup> Cited from ROSENOW: *Loc. cit.*

<sup>3</sup> LOEB: Archiv für Entwicklungsmechanik. 1898, vii, p. 631.

<sup>4</sup> LOEB: Archiv für die gesammte Physiologie. 1904, ci, p. 340.

<sup>5</sup> MATHEWS: This journal, 1903-4, x, p. 319.

## V. EFFECT OF LÆVULOSE.

It is to be assumed from the work of Maze<sup>1</sup> that lævulose may act as a depolarizer in the process of respiration, and permit oxidation to

TABLE V.  
FUNDULUS INJECTED WITH  $\frac{1}{2}m$  LÆVULOSE.

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
71	hrs. min. 10	hrs. min. 1 45	4	3	5	2
72	15	1 28	6	3	7	2
73	10	2 35	1	3	2	2
116	1 20	2 40	6	3	7	2
117	10	2 30	7	3	2	8
120	5	3 15	7	3	7	3
121	28 00	5 00	3	6	3	6
125	50	3 30	9	1	6	4
127	1 40	4 00	10	5	9	6
128	19 10	4 20	2	2	2	2
129	28 00	3 25	4	6	3	7
130	4 00	3 15	3	3	4	2
131	3 45	3 15	6	4	5	5
132	3 45	3 15	6	4	5	5
133	28 55	4 00	1	9	9	1
Total . . . . .			75	58	76	75

## SUMMARY.

Alive, 151 . . . . . Controls, 75 (50%)    Injected, 76 (50%)  
Dead, 115 . . . . . "    58 (50%)    "    57 (50%)

go on in the absence of atmospheric oxygen. It was hoped that increasing the lævulose content of the blood of *Fundulus* would render them more resistant to lack of oxygen. The results of the experi-

<sup>1</sup> MAZE: *Loc. cit.*

ments so far do not seem to bear out this conclusion. These results are given in Table IV.

The animals were injected with a  $\frac{5}{8}m$  solution of lævulose as being approximately isotonic with the blood. From the summary it will be seen that approximately the same number of controls and injected animals remained alive and died. Lævulose is thus apparently indifferent in its action in regard to lack of oxygen. The experiments were necessarily brought to a close before the author was satisfied in regard to this, and further experiments will be taken up another summer. Varying strengths of lævulose should be tried. It may be also that not enough time is allowed for the absorption of the lævulose into the blood stream from the body cavity. Experiment No. 133, which was the last experiment tried, and which taken by itself is a very striking result, seems to indicate such a possibility. In this experiment 29 hours elapsed between the time of injection and the time of placing in the experiment.

#### SUMMARY.

1. Increasing the alkalinity of the blood of *Fundulus heteroclitis* by the injection of three to eight drops of  $\frac{5}{16}m$  solution of sodium bicarbonate increases their power of resistance to lack of oxygen. Decreasing the alkalinity by the injection of  $\frac{m}{250} - \frac{m}{500}$  solution of acetic acid decreases their power of resistance.

2. Increasing the lævulose content of the blood seems to have no effect on the power of resistance to lack of oxygen.

These observations were made in the Marine Biological Laboratory at Woods Hole, in a room provided by a grant from the Carnegie Institution, to whom and to the Assistant Director, Dr. F. R. Lillie, my thanks are due.

I wish also to thank Professor E. P. Lyon for suggestions and criticism, and I am especially indebted to Professor A. P. Mathews, under whose direction the work was done.

## THE PHYSIOLOGY OF THE DIGESTIVE TRACT OF ELASMOBRANCHS.

By M. X. SULLIVAN.

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Laboratory of Comparative Anatomy, Brown University.]

SIXTEEN per cent of the dogfish taken at the laboratory of the Bureau of Fisheries, Woods Hole, Mass., during the summer of 1904, were found to contain lobsters; 34.17 per cent rock crabs, and 20.1 per cent spider crabs. The carapace of these organisms consists of salts and chitin, which is highly resistant to reagents. As the carapace was found in varying degrees of decomposition, and further since the carapace of crabs and lobsters fed to the fish could not be found in the stomach after four days of digestion, the question arose as to whether the dogfish really did digest chitin. Accordingly, during this summer I began a physiological study of the alimentary canal of *Mustelus canis*, and extended the work to include the sand shark (*Carcharias littoralis*) and the spiny dogfish (*Squalus acanthias*).

The experiments of 1904 consisted in the study of the normal contents of the stomach and the spiral valve; in the determination of the digestive power of the juices contained in the stomach and spiral valve; in the preparation for artificial digestion of extracts of buccal, esophageal, gastric, and intestinal mucous membranes, and of the extracts of the pancreas; and finally in determining whether or not these fishes digested chitin.

<sup>1</sup> During the summer of 1905 the work of the previous summer was reviewed, amplified, and extended to include the torpedo (*Tetronarce occidentalis*), the dusky shark (*Carcharhinus obscurus*), the skate (*Raja erinacea*), and the mackerel shark (*Lamna cornubica*). More attention was paid to the physiology of the pancreas, middle intestine, and spleen; and histological preparations were made of the various parts of the alimentary canal from the esophagus to the anus. The résumé of the work is as follows:

(A) **Buccal cavity.** — These fish as a rule swallow their prey whole without mastication. Naturally, we should suppose that the buccal mucus has little digestive action. This probability is increased by the absence of glands. The buccal mucus consists of epithelium and connective tissue. There are several layers of epithelium. Next to the connective tissue are cylindrical cells. Above these cells are several layers of mucous cells. The superficial epithelium consists of oval or irregularly polygonal cells. Extracts of buccal mucus have no digestive action on starch, fibrin, or fat.

(B) **Esophagus.** — The reaction of the esophagus is often acid, probably due to regurgitation from the stomach. Extracts of esophageal mucus of the fasting fish have no digestive action. The mucus contains ciliated cylindrical cells and goblet cells.

(C) **Stomach.** — The stomach of elasmobranchs consists of a large cardiac sac and a narrow pyloric tube.

*Cardiac sac.* — An analysis of the contents of the sac of *Mustelus canis*, *Carcharias littoralis*, *Squalus acanthias*, *Tetronarce occidentalis*, *Carcharhinus obscurus*, *Raja erinacea*, *Lamna cornubica*, *Galeocerdo tigrinus*, showed as a rule syntonin, proteoses, and peptones. Occasionally in the stomach contents no peptones could be found. Using phenolphthalein, alizarin, and dimethyl-amido-azobenzol, as indicators, experiments were made to determine —

1. The total acidity of the stomach contents in terms of hydrochloric acid.
2. The physiologically active hydrochloric acid.
3. The free hydrochloric acid.

The results are given in the table on page 44.

The acidity of the stomach contents depends on the period of digestion, the fasting stomach being practically neutral, and upon the nature of the food, the greatest acidity being found when the stomach was full of partly digested lobsters and crabs.

Glycerin-hydrochloric acid extracts of the mucus of the cardiac sac digest fibrin at 20° C., but better at 38° C., with the formation of syntonin, proteoses, and peptones.

In the cardiac part of the stomach we can distinguish superficial epithelium, the lumen of the peptic glands, and the glandular epithelium in the crypts. The superficial epithelium consists of pyramidal cells. The peptic glands begin just behind the esophagus and extend to the pylorus, with the deepest glands in the centre. Each gland is a cylindrical tube. The epithelium of the neck of the glands consists



of cylindrical cells, while the cells of the body of the glands are regularly polygonal, highly granular, and closely packed together. Only this one kind of cell can be distinguished in the body of the gland.

*Pyloric tube.*—The pyloric tube has no digestive function. The superficial epithelium is like that of the stomach. The glands are short, and the polygonal peptic cells are absent.

(D) **Middle intestine.**—Extracts of the middle intestine or duodenum of *Mustelus canis* and *Carcharias littoralis* show no digestive action. The epithelium consists of cylindrical cells and goblet cells.

Species.	Total acidity in percentage hydrochloric acid.	Physiologically active hydrochloric acid, average percentage.	Highest percentage free hydrochloric acid.
<i>Mustelus canis</i> . . . . .	{ 0.04-1.00 Aver. 0.73 50 individuals	0.538 6 individuals	0.2
<i>Carcharias littoralis</i> . . . .	{ 0.1-1.2 Aver. 0.87 25 individuals	0.614 10 individuals	0.31
<i>Squalus acanthias</i> . . . . .	{ Aver. 0.67 60 individuals	No tests	No tests.
<i>Carcharinus obscurus</i> . . . .	{ Aver. 0.55 2 individuals	0.493 2 individuals	0.254
<i>Lamna cornubica</i> . . . . .	{ 0.275 1 individual	0.229 1 individual	0.172
<i>Galeocerdo tigrinus</i> . . . . .	{ 0.93 1 individual	0.812 1 individual	None.
<i>Tetronarce occidentalis</i> . . .	{ 0.51 1 individual	No tests	None.

(E) **Spiral valve.**—The arrangement histologically is like that of the middle intestine. Extracts of the spiral valve show no digestive action.

(F) **Pancreas.**—Extracts of the pancreas vary in their action. Some have no digestive action whatever. Neither water extracts nor sodium carbonate extracts of the pancreas of the various elasmobranchs have any digestive action on hard-boiled egg or fibrin. Extracts of the middle intestines do not activate the pancreas. Bile activates the pancreatic extracts slightly. The spleen activates the pancreas most. A boric acid extract of the pancreas, plus a boric extract of the spleen of a fish in full digestion, gave the greatest digestion of fibrin. Pancreatic fistulas were of little value. Of six made, but one gave a juice having any digestive action on fibrin, and even this was slight.



Analysis of the contents of the spiral valve showed leucin and tyrosin, proving that the pancreas acts in these animals as in the higher animals. The fresh pancreas emulsifies olive oil. Glycerin-acetic extracts of *Mustelus canis* convert starch to sugar. None of the standard extracts of the pancreas of *Carcharias littoralis* and *Raja erinacea* showed the amylolytic ferment.

(G) **Rectal gland.**—The rectal gland is a compound tubular gland. Extracts of this gland do not show any digestive activity.

(H) **Action of gastric juice on chitin.**—The natural juice of the stomach of *Mustelus canis* digested fish and fibrin in vitro with the formation of syntonin, proteoses, and peptones, but did not digest the carapace of lobsters and crabs. The frequent change of the gastric juice and much trituration, however, softened the shells and broke them up into a fine mass such as may be found in the spiral valve of the fish. The chitin is not regurgitated. Pieces may be found intact in the spiral valve. These fishes do not derive nourishment from the carapace, but dissolve out the salts by means of the relatively strong acid which collects in the stomach, and triturate the chitinous mass into extremely fine particles. This finely divided mass is excreted.

# THE PHYSIOLOGY OF CELL-DIVISION. — I. EXPERIMENTS ON THE CONDITIONS DETERMINING THE DISTRIBUTION OF CHROMATIC MATTER IN MITOSIS.

By RALPH S. LILLIE.<sup>1</sup>

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## INTRODUCTORY.

LIVING matter, as Graham<sup>2</sup> was the first to emphasize, is invariably characterized by richness in colloid substances. Colloids in fact form the basis of cell-structure; that is, they form, in distinc-

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<sup>2</sup> GRAHAM: Philosophical transactions, 1861, cli, p. 183.

tion to the water and the soluble crystalloid portion of the cell-constituents, a comparatively coherent and permanent and definitely arranged substratum by which the distribution of the other substances is largely determined. Hence in accounting for protoplasmic structure *i. e.*, for the special mode of distribution shown by the different substances in the cell, it is necessary first of all to consider the conditions that determine the peculiar disposition exhibited by the colloids.

**Relation of colloids to cell-structure.** — The first question then to be asked is this : What are those special properties of colloids on which depends the possibility of their forming the definite structural arrangements observed in cells ? These may be regarded as essentially two in number : first, their tendency to form aggregates and coherent systems of various kinds (gelation, coagulation, changes in state of aggregation generally) ; and, second, the electrically charged nature of the colloid particles themselves, a property which leads adjacent colloid particles or their aggregates either to repel or attract one another electrostatically (according to whether their charges are of the same or opposite sign). On the first property depends the formation of the various colloid aggregates in the cell and the particular structure of these ; the second determines in large part — as I propose to show below — the relative disposition assumed by the aggregates within the cell — for example, the arrangement of the nuclear aggregates or chromosomes during cell-division. The two properties are closely inter-connected, for changes in the state of aggregation are apparently dependent on changes in the electrical condition of the particles ; thus coagulative changes in a hydrosol are to be regarded as due to the coalescence of colloid particles that have lost either the whole or a portion of their mutually repellent surface-charges ; adjacent particles may then come in contact and coalesce under the influence of their surface-tension, which increases as the surface charge diminishes,<sup>1</sup> and tends to minimize the surface of contact between colloid and medium. The mutual repulsion of the particles appears indeed to be one of the chief factors on which the stability of a colloid system depends, and changes in this condition may lead to changes in the state of aggregation and so, in the cell, to changes in protoplasmic structure. It seems convenient, however, for present purposes, to regard (1) the formation of definite aggre-

<sup>1</sup> For a discussion of the relation of this change to coagulation see BREDIG : *Anorganische Fermente*, Leipzig, 1901, pp. 14 *et seq.*

gation-states and (2) the electrostatic action of the charges on separate aggregates as distinct phenomena and to assign to each a definite rôle in the cell-processes, determining respectively the structure and the distribution of the colloid aggregates in the cell.

**Formation of colloid aggregates in cells.**—One physiologically important peculiarity of certain colloids, consideration of which throws light on the nature of their relation to protoplasmic structure, is the tendency which their particles exhibit to form coherent arrangements of a more complex kind, presenting often a net-like or filamentous configuration. A change of this kind is the determining condition of gelation, a phenomenon which Hardy<sup>1</sup> has shown to be due to a coalescence of colloid-particles into a network-like or foam-like arrangement. It is probable that such formations are of particular importance in protoplasm, since a network-like and especially a foam-like or alveolar structure appears to exist in many cells.<sup>2</sup> Thread-like or filamentous formations are also highly characteristic of many of the colloids of the cell, especially the nucleo-proteid or chromatin portion of the nuclear colloids during mitosis (chromatic filaments and chromosomes). This peculiar structure appears frequently to be due to a serial arrangement of smaller rounded colloid-particles,<sup>3</sup> due to conditions whose nature is obscure at present but possibly connected with an electrical polarization of adjacent particles causing their end-to-end union.<sup>4</sup> Changes in the aggregation-state of the protoplasmic colloids must also be of importance in cell-processes. Such changes, according to the theory cited above, must occur whenever the surface-charge on the colloid particles is altered in any way, decrease of sur-

<sup>1</sup> HARDY: Proceedings of the Royal Society, 1900, lxvi, p. 95.

<sup>2</sup> Cf. BÜTSCHLI: Untersuchungen über mikroskopische Schäume und Protoplasma, Leipzig, 1892.

<sup>3</sup> Cf. HERMANN: Archiv für mikroskopische Anatomie, 1891, xxxvii, Plate 31, Fig. 13, etc. (spireme from a spermatocyte of *Proteus*); BRAUER: *Ibid.*, 1893, xlii, Plate 11, Figs. 30-50 (germ-cells of *Ascaris*). The composition of the chromatic filaments by serially arranged rounded particles is particularly distinct in these figures. Other examples could be given.

<sup>4</sup> I have made this suggestion elsewhere (Biological bulletin, 1903, iv, p. 164) to account for the formation of the astral radiations in cells. In GALLARDO'S experiments with quinine particles suspended in turpentine the particles arrange themselves in rows along the lines of force. So also in FARADAY'S similar experiment with small pieces of silk. If such particles were to cohere, filaments would be formed. See GALLARDO: "Interpretación dinámica de la división celular," Buenos Aires, 1902. Other filamentous arrangements of colloid particles in cells may possibly thus be formed.

face or aggregation<sup>1</sup> (a change leading in the direction of coagulation) resulting when the density of the charge is diminished, and enlargement of surface, *i. e.*, still finer subdivision of the particles, following an increase in density.

The degree of this aggregation or coalescence of particles may vary greatly according to conditions. It seems probable indeed that slight and reversible changes in the aggregation-state of the colloids are of constant occurrence in living protoplasm, and form the condition of many typical processes. Thus I have obtained evidence (shortly to be published) that the contraction of the swimming plate in *Ctenophora* is accompanied and probably conditioned by a change of this nature, and if this is true it may be considered as almost certain that many other, perhaps all, contractile processes are similarly conditioned. Again — as another possible instance of the importance of such changes — oxidations and reductions, processes that are of constant occurrence in protoplasm, imply respectively the withdrawal or addition of negative charges (or electrons) relatively to the substances acted on; and it is therefore obvious that when these form the colloid portion of the cell aggregation-changes must follow. Variations in the surface-extent of the reacting groups of substances, and so in the rate of chemical action, will be one result of this.<sup>2</sup>

**Attractions and repulsions between colloid aggregates.** — The influence of electrostatic attractions and repulsions is seen in the relative positions adopted by many characteristic structures in the cell. Certain colloid aggregates, notably the chromosomes and chromatic filaments of the dividing nucleus, exhibit an arrangement which suggests strongly that mutual repulsion may be the controlling factor in determining their relative positions.<sup>3</sup> Now it is certain that colloid aggregates do actually attract or repel one another electrostatically; the proofs of this may be summarized as follows: first, the colloid par-

<sup>1</sup> Supposedly this will continue until the remaining charge, now spread over a smaller surface, attains sufficient density to prevent further aggregation. A new condition of equilibrium is then reached.

<sup>2</sup> Compare MATHEWS: This journal, 1904, x, p. 292.

<sup>3</sup> Professor BOVERI has kindly drawn my attention to the following paper in which also the distribution of the chromosomes in abnormal monaster figures in eggs of *Echinus* (fertilized by sperm of *Strongylocentrotus*) is ascribed to a mutual repulsion. M. BOVERI: *Jenaische Zeitschrift für Naturwissenschaft*, 1902-03, xxx: on p. 426 the author refers to the peculiar distribution of the chromosomes in the following terms: "eine Erscheinung die nur als gegenseitige Abstossung, sei sie im übrigen vermittelt wie sie will, bezeichnet werden kann."



ticles in any colloidal solution carry electric charges of a definite sign, positive or negative according to the nature of the colloid and of the medium; their movement in the electric field demonstrates this, and their mutual repulsion follows as a necessary corollary since all similarly charged bodies repel one another. Second, the fact that many hydrosols are stable so long as their particles are charged (*i. e.*, exhibit electrical migration), but coagulate when the charge is neutralized, indicates that the charges on the particles prevents their union; this again indicates a mutual repulsion. Third (the reverse of the second case), colloidal solutions whose particles are of opposite sign (*e. g.*, arsenious sulphide and ferric hydroxide) coagulate one another<sup>1</sup> when mixed, proving that the colloidal particles in the two solutions unite to form larger aggregates; this indicates a mutual attraction between the oppositely charged particles. Precipitation of a colloidal solution by ions of opposite sign to the colloid particles is a phenomenon identical in some respects with this last.

It is clear therefore that electrostatic attractions and repulsions between the colloid particles or aggregates — in other words, the electrostatic distance-energies of the particles — must be a factor of greater or less importance in determining the relative positions which these structures adopt within the cell. In certain instances this factor appears to be of predominant importance, especially with the chromatic filaments and chromosomes, as will be shown below. It is evident, however, that the disposition of other colloid groups in the cell must be subject to similar influences. Any charged particle in the cell must, in fact, be regarded as seeking a position of electrostatic equilibrium.

#### EXPERIMENTAL. USE OF MAGNETIC CHROMOSOME-MODELS.

The influence of mutual repulsion in the arrangement of the structural units of the cell is best seen in the nuclear colloids, *i. e.*, the chromatin, especially during mitosis. These colloids are pronouncedly acid in their general chemical character,<sup>2</sup> and are hence easily and sharply differentiable *in situ* by the use of basic dyes; their relative positions in the cell and the changes in these can therefore be determined with ease and great precision. It can hardly be

<sup>1</sup> BILTZ: Berichte der deutschen chemischen Gesellschaft, 1904, xxxvii, p. 1095.

<sup>2</sup> For a general account of nucleo-proteids and nucleins, see COHNHEIM: Chemie der Eiweisskörper, 2te Auflage, Braunschweig, 1904, pp. 219 *et seq.*

doubted that the existing descriptions of the mitotic process, though based chiefly on the study of fixed and stained preparations, represent in an essentially accurate manner the true disposition and movements of these colloid aggregates in the living cell. The remarkable uniformity of these descriptions is in itself a sufficient proof of this. We may therefore conclude that the use of appropriate fixing fluids coagulates the colloids without producing any material alteration in their disposition and relative positions.<sup>1</sup>

The above hypothesis that the relative positions of the chromatic filaments and chromosomes are due largely to mutual repulsion can be tested by making use of similarly shaped artificial structures that possess this property, and by determining if under certain conditions, especially subjection to a centrally attractive force, these form arrangements similar to those shown by the chromatin aggregates. If an identity of arrangement is shown, the above view must receive strong confirmation. We may take as the simplest case the example of a cell containing in its equatorial plate a relatively small number

<sup>1</sup> In a number of other cell-structures the same arrangement of the colloids in radiating figures, parallel threads, or spireme-like groups is found as in the nucleus. It must be assumed that electrostatic attractions and repulsions between colloid aggregates play an important part not only in the nucleus but elsewhere in the cell in determining the disposition of the colloids and hence the type of structure presented. Cf. v. BERGEN: "Strukturbilder, Netzapparate, Saftkanälchen, Trophospongien im Protoplasma verschiedener Zellenarten," *Archiv für mikroskopische Anatomie*, 1904, lxiv, p. 498. Plate 29, Figs. 1-4, shows spireme-like structures in the cytoplasm of spinal ganglion-cells of rabbits (this is the "apparato reticolare interno" of GOLGI, a network of fine threads colored black by osmium): Fig. 8 shows a similar structure in a ganglion-cell of the chick, with a parallel arrangement of fibrils. Similar structures are found also in ciliated cells, pancreas cells, cartilage cells, interstitial cells of testis, etc. Such structures seem common in secretory cells; see GURWITSCH: *Morphologie und Physiologie der Zelle*, pp. 176 *et seq.* for descriptions and figures of such formations. They have frequently the form of bundles of parallel fibrils, *e.g.*, in pancreas-cells (MATHEWS), cells of salivary glands (GARNIER), milk glands (LIMON), digestive gland of crayfish (VIGIER), intestinal cells of frog (HEIDENHAIN). Short parallel rods are also figured from an embryo-sac cell of the lily (P. and M. BOUIN). Somewhat similar structures are described by CARNOY and LEBRUN in the germinal vesicle of Batrachia (La cellule, 1898, xiv, p. 113, and 1900, xvii, p. 203); the nucleoli resolve themselves into groups of slender filaments which either centre to a point forming a radiating figure, or are attached at intervals along an axial thread from which they stand out at right angles and parallel to one another (xiv, Plate 13, Figs. 8-11, etc., xvii, Plates 1, 2, Figs. 13-17, etc.). arrangements indicating mutual repulsion of the filaments.



of rounded chromosomes at approximately equal distances apart.<sup>1</sup> On the above view the intervals between adjacent chromosomes are an expression of their mutual repulsion. Can such a system of mutually repellent units disposed at almost equal intervals in a single plane be simulated by means of artificial models?

**Methods.**—Some years ago the use of Alfred Mayer's floating magnets<sup>2</sup> suggested itself to me as affording a possible means of answering this question. Equally magnetized needles<sup>3</sup> are passed through small cubes or discs of cork and floated with all like poles uppermost on the surface of some supporting liquid. Such needles exhibit a mutual repulsion due to the proximity of like magnetic poles; each needle thus constitutes a freely moving unit which repels all other similar units. If several such are placed close together on the water-surface, they immediately recede from one another, rapidly at first, then more slowly since the repelling force follows the usual law of diminution with inverse squares. If, now, the needles are exposed to the action of a bar-magnet held vertically with its attractive pole a short distance above the floating group of needles, the latter approach one another until their mutual repulsion balances the centrally directed component of the attractive force; the

<sup>1</sup> This arrangement is frequent, as may readily be seen in examination of the cell-division figures in cytological papers. The following figures show polar views of equatorial plates with this typical arrangement of chromosomes: VOM RATH: *Archiv für mikroskopische Anatomie*, 1892, xl, p. 102, Plate 5, Figs. 8, 17, 24, 25 (germ-cells of cricket); *Ibid.*, 1895, xlvi, p. 168, Plate 6, Figs. 7, 8 (germ-cells of cricket), 16, 22 (germ-cells of frog); *Zeitschrift für wissenschaftliche Zoologie*, 1899, lvii, p. 97, Plate 7, Figs. 12, 14 (spermatocytes of salamander); BRAUER: *Archiv für mikroskopische Anatomie*, 1894, xliii, p. 162, Plate 8, Figs. 2, 3, 4, 5, 12 a (egg of *Artemia salina*); HENKING: *Zeitschrift für wissenschaftliche Zoologie*, 1891, li, p. 685, Plate 35, Figs. 6, 10, 31, 33-36 (germ-cells of *Pyrochoris*), Plate 36, Fig. 124 (germ-cells of *Pieris brassicæ*); *Ibid.*, 1892, liv, p. 1, Plate 6, Figs. 146, 148, 149, 161, 164, 165, 182, 183, 192, 193 (polar views of equatorial plates in germ-cells of other insects); BOVERI: *Jenaische Zeitschrift für Naturwissenschaft*, 1887, xiv, p. 423, Plate 28, Figs. 12, 22 (egg of *Ascaris lumbricoides*); VAN DER STRICHT, *Archives de Biologie*, 1895, xiv, p. 243, Plates 20, 21, Fig. 6, etc. (egg of *Amphioxus*); CALKINS: *Journal of morphology*, 1895, xi, p. 271, Plate 17, Fig. 7, similar figures on Plate 18 (germ-cells of earthworm); GRIFFIN: *Journal of morphology*, 1899, xv, p. 711, Plate 33, Fig. 40 (egg of *Thalassema*). The above references are to a wide range of forms. Numerous other examples can be found in the literature.

<sup>2</sup> See any text-book of Physics, *e. g.*, GANOT's 15th ed., 1901, p. 721.

<sup>3</sup> Any desired number may be magnetized by placing in the interior of a coil (*e. g.*, of a dynamo) through which a current is passing.

result is the production of regular figures of remarkable beauty and symmetry (Fig. 1). The resemblance of these arrangements to equatorial plates of the above-described kind is extremely close, and implies that the essential determining conditions are identical in the two cases.

**Comparison with conditions in cells.**— Exactly in what does this identity consist? In the system of floating magnets, the final disposition of the units depends on three conditions: (1) the mutual repulsion of the individual units; (2) the centripetal attractive force due to the bar-magnet; and (3) the confinement of the movement to a single plane, that of the water-surface. The stationary position is one of equilibrium between the attracting and the repelling forces.

In what manner are these conditions represented in the dividing cell? With respect to the first, the mutual repulsion of the chromosomes, we find this to be conditional on the colloid nature of these structures. Corresponding to the second condition, there must be an

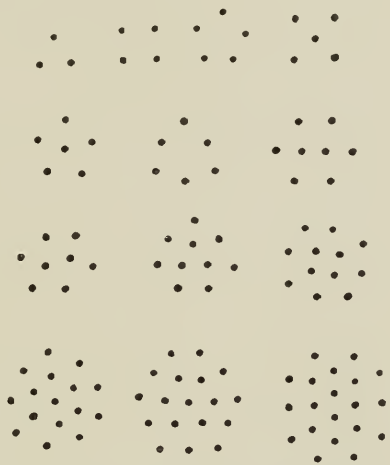


FIGURE 1. — Configuration of groups of 3, 4, 5, 6, 8, 10, 12, 16, 18, and 20 floating magnets.

influence in the cell that attracts the chromosomes to a central position midway between the astral centres. As to the nature of this influence, we may with some fair degree of justification regard it as probably of the same kind as that which keeps the nucleus in its usually central position in most cells. During mitosis the chromosomes as a rule occupy the centre of the cell; in such cases this coincides with the region midway between the two astral centres. But not infrequently the dividing nucleus is excentric in position, as in unequally cleaving eggs or the maturation divisions of all eggs. The chromosomes, nevertheless, always adopt at the metaphase a position midway between the two astral centres. The question therefore is: why do the chromosomes adopt a median position relatively to the two centres of radiation, whatever the position of the latter in the cell?

The segregation of the nucleo-proteid material of the cell in a more

or less centrally situated aggregate, which is usually separated from the cytoplasm by a membrane,<sup>1</sup> is in reality the distinguishing feature of cell-organization, and, as we now know, it is extremely wide-spread and indeed almost universal in organisms. The explanation of this remarkable condition is, however, as yet uncertain. It probably depends in some manner on the strongly marked electro-negative characteristics of the nuclear colloids. That the chromatin-aggregates are negatively charged follows directly from their acid properties which, though most strongly marked at the time of mitosis, are always more or less evident. The readiest index of this acidity is the characteristic property of combining strongly with basic dyes to form deeply colored compounds.<sup>2</sup> This acidity implies electrical negativity: liberation of hydrogen-ions, the property on which all acidity depends, means that the colloid aggregate is left with a residual negative charge—in other words, that it corresponds to an anion or an aggregate of anions, and possesses the electrical properties of these. The negativity of the chromatin-aggregates must be greatest at the time of mitosis, since then their acidity is most marked. At this stage they are in all probability similar chemically to the nuclear material of sperm-heads, which consist chiefly of nucleins, compounds intermediate in many respects between nucleoproteids and nucleic acid, and much more strongly acid than the former.<sup>3</sup> To this greater degree of acidity will correspond a more strongly marked negativity.

It seemed possible at first that the central position of the nuclear colloids in the cell might be due to an attraction on the part of an

<sup>1</sup> It is possible that the formation of this membrane also depends on the strongly electro-negative character of the nuclear colloids, and that it is essentially a precipitation-membrane produced by the interaction of nuclear and cytoplasmic colloids at the boundary between the two. On this view it should possess the common property of colloid membranes (parchment, etc.) of being permeable to most crystalloids but not to colloids. Such a membrane would therefore prevent direct interaction of the two groups of colloids while permitting a free interchange of soluble crystalloid constituents and dissolved gases. Its dissolution at mitosis remains unexplained; possibly the doubling of nuclear material during and as a result of this process is dependent on the circumstance that then the colloids of nucleus and cytoplasm are brought in direct contact for a time. At least this last is a periodically recurring condition that always accompanies cell-division and growth.

<sup>2</sup> Cf. HEIDENHAIN: *Archiv für die gesammte Physiologie*, 1902, xc, p. 115; *Ibid.*, 1903, xcvi, p. 440.

<sup>3</sup> Cf. COHNHEIM: *Loc. cit.*, pp. 210 *et seq.*

electro-positive system of cytoplasmic colloids,<sup>1</sup> since in tissues fixed by the usual methods the nuclear colloids stain in basic and the cytoplasmic in acid dyes, indicating a contrast in the sign of the charge borne by their respective particles. But it should be recognized that the action of acid fixing fluids would render the proteids more strongly electro-positive than they were in life, and might even reverse the sign of the charge in particles that were originally not strongly electro-negative.<sup>2</sup> It appears, in fact, that the proteids of the cell-body are neither pronouncedly acid nor pronouncedly basic, but have the amphoteric properties of most simple proteids. They appear to be chiefly globulins and myosin-like bodies with some albumins and iron-containing nucleo-albumins.<sup>3</sup> In life, if the reaction of the protoplasm were alkaline, their charges would be chiefly negative, although under certain conditions (as excessive production of carbonic or other acid) they might conceivably become positive. As a fact, the only dyes which do stain the colloids of living protoplasm (intra-vitam stains) are basic<sup>4</sup> (neutral red, etc.) indicating that the particles are prevailingly negative. The above explanation must therefore be rejected for this as well as for certain other reasons. The nuclear colloids on the contrary possess well-marked acid properties, and their reaction is not reversed by the action of fixing agents. They must therefore be far more strongly electro-negative than the cytoplasmic colloids; and their central position probably depends, in some manner not more precisely definable at present, upon this strongly negative character. It may be that the central regions of most cells are more electro-positive than elsewhere; this, however, is at present mere conjecture.

But with respect to the attraction of the chromosomes to the inter-astral area during mitosis — a phenomenon of striking constancy and uniformity — a definite and probably essentially correct explanation may be given. The repulsion of the chromosomes from the astral centres<sup>5</sup> indicates that these regions are strongly negatively charged.

<sup>1</sup> R. LILLIE: This journal, 1903, viii, p. 273.

<sup>2</sup> Cf. HARDY'S experiments on the reversal of the sign of proteid colloid particles by the action of acid (Journal of physiology, 1899, xxiv, p. 288; also *Ibid.*, 1903, xxix. Proceedings of the Physiological Society, p. xxvi). For a simple explanation of this action see J. LOEB: University of California Publications, Physiology, 1904, i, p. 149. An identical explanation is given by J. BILLITZER: Zeitschrift für physikalische Chemie, 1903, xlv, p. 329.

<sup>3</sup> Cf. COHNHEIM: *Loc. cit.*, p. 200.

<sup>4</sup> Cf. MANN: Physiological histology, Oxford, 1902, p. 410.

<sup>5</sup> For evidence of this see below, pp. 77 *et seq.*

If this is true, it is clear that the corresponding positive charges must exist in neighboring regions of the cytoplasm. In other words, the astral centres can acquire their additional negative charges only by the withdrawal of negative charges (or electrons) from the adjoining cytoplasmic areas which would thus become positive. It seems further clear that this effect would be greatest in the region midway between two adjoining astral centres, since there the two actions would be superposed on one another. This region would therefore be strongly positive to a degree corresponding to the negativity of the astral areas; and negatively charged bodies would hence be attracted electrostatically toward it. The chromosomes being negatively charged aggregates are thus attracted to a positive region midway between the astral centres. There is no doubt that they are so attracted; and the two chief conditions determining their distribution, viz., their repulsion from the asters combined with attraction toward the interastral area, become at once intelligible on this hypothesis. The exact nature of the chemical changes that lead to the formation of these localized electro-negative areas need not concern us here.<sup>1</sup>

With respect to the third peculiarity of the equatorial plate stage — the arrangement of the chromosomes in a single plane — it is clear that this position is one of equilibrium, and that the chromosomes are free to move in the plane perpendicular to the spindle axis and midway between its poles, but that some condition prevents their movement toward the poles. They therefore repel one another along this plane and adopt side by side positions within it. The condition resisting their movement toward the poles appears to be a strong repellent action exercised by the astral areas on the chromosomes. I shall show later that on this view the typical aggregation in a single plane is readily to be accounted for, and that it can be simulated experimentally with floating magnets and a combination of a centrally situated attractive pole with two laterally placed repellent poles.<sup>2</sup>

**Configuration of equatorial plates in cells.** — When we turn to the representations of the equatorial plates of dividing cells, we observe a great variety of configurations. The simplest arrangements are seen

<sup>1</sup> Professor MATHEWS has suggested that the astral centres may be areas where active reduction-processes are in progress; this would imply the addition of negative charges. The chemical basis of mitosis would on this view be of a respiratory nature.

<sup>2</sup> See pp. 77 *et seq.*



when the chromosomes are rounded in shape; then the shape of the individual chromosome plays no important part, and the configuration of the groups is often very similar to that of the above-described systems of isolated floating magnets. Some of the arrangements are shown in Fig. 1. The number of possible configurations becomes greater than one when the mutually repellent units exceed four in number, and as the number of units increases, the number of configurations for any given number increases also. In dividing cells with a large number of small chromosomes, the individual units are frequently found to be distributed in the equatorial plate in a manner apparently identical with that shown by corresponding groups of floating magnets, *i. e.*, at almost equal distances apart over the entire area of the plate. This appearance is in fact very frequent in dividing cells.<sup>1</sup>

When, on the other hand, the chromosomes are elongated, looped, or of other definite shape, conditions become more complex. Then the individual repellent particles are connected together into coherent aggregates of definite form; their relative positions in a single aggregate are fixed; the aggregate as a whole, however, is free to move. We have in this case to deal with mutually repellent aggregates of a certain form, and it is found that this form, as such, has a marked influence on the relative positions assumed by the aggregates with reference to one another. For instance, such aggregates, when of linear form, exhibit a tendency to dispose themselves side by side and parallel under the influence of an attractive force. This is readily shown by the use of rows of floating magnets. Any number of these may be connected in a linear group by means of a thin, straight wire traversing the supporting corks. If two such aggregates of equal length be subjected as above described to the action of an attractive magnetic pole, they are found to range themselves side by side and parallel, *i. e.*, at equal distances apart throughout their entire length. Three such aggregates exhibit similar behavior, provided their distance apart is small compared with their length, otherwise they form angles with one another (Fig. 3). When the number of

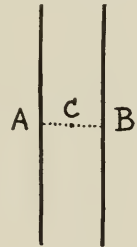


FIGURE 2. — Position assumed by two straight linear, mutually repellent aggregates under action of attractive force. *C*, centre to which attractive lines of force converge (projection of attractive pole on water-surface). *A* and *B*, centres of attraction of the two aggregates.

<sup>1</sup> See footnote, p. 52.

aggregates is greater than three, a variety of conditions enter that disturb this tendency to parallelism, though it still remains more or less evident, especially when the aggregates are brought close together.

**Parallelism of linear aggregates.**—We shall now consider more closely the conditions on which these arrangements depend. The

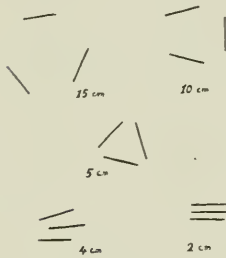


FIGURE 3. — Relative positions assumed by group of three rod-like aggregates with varying intensity of attractive force. (Each aggregate in this and the following groupings (Figs. 3-9) is 30 mm. in length and consists of 9 equidistant floating magnetized needles connected by a thin wire. The attraction is due to a large bar-magnet 250 mm. in length; the distances (15 cm., etc.) indicate the height of the attractive pole above the water-surface in each grouping.)

case of two equal linear aggregates is the simplest, and parallelism with the extremities of the aggregates perpendicularly opposite is found to be the position of stable equilibrium (Fig. 2). Then all the mutually repellent particles are so placed that the distance of each from its nearest neighbor in the other filament is the same in all cases; this distance is an index of the repellent force necessary to compensate the centripetal tendency due to the centrally directed attractive component.

In a complete explanation of the phenomenon it is necessary to bear in mind first that each particle in the one filament is repelled by *all* the particles in the other, and hence with an intensity and in a direction that vary according to its position in the filament; and, second, that the attractive force, which acts along lines converging toward a central point, also acts differently, with respect to both direction and intensity, on each particle. It is found experimentally that equilibrium prevails between the attracting and repelling forces when the two filaments are opposite and parallel and a certain distance apart. Then — to consider first the conditions of attraction — the centres of each filament are equidistant from the point toward which the attractive lines of force converge (*C*, Fig. 2) and in a straight line with it, and the attractive lines of force are similarly and symmetrically distributed with reference to both filaments. A similar symmetry in the distribution of the repellent forces between the two filaments exists also in this position. The repellent action of each filament acts most strongly at the central point of the other, and progressively diminishes from this point toward the extremities; this diminution is



symmetrical on either side of the central point of the filament. There is then in the position of parallelism a symmetry in the disposition of both attractive and repellent forces. The intensity of the repulsion increases more rapidly than the attraction as the filaments approach the centre; hence there is at a certain distance apart a position where repulsions balance attractions, and a stationary condition results.

**Arrangements shown by groups of more than two linear aggregates.**

—When the number of linear aggregates in the system is greater than two, conditions become more complex. The position of any one aggregate depends on the repulsion from all the others, the preponderant influence being exercised by those immediately adjoining it. This last condition produces a tendency to a parallelism of immediately adjacent filaments which increases as the distance separating the filaments becomes less; this is seen in Figs. 3 to 9. As a rule, however, the relative positions of any two filaments in a group of several are so affected by the action of the others that the parallelism at best is only approximate.

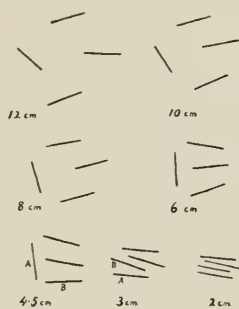


FIGURE 4.—Grouping of four rods with gradually increasing attraction.

Figs. 3 to 9 represent accurately a series of arrangements exhibited respectively by groups of three, four, five, six, eight, and ten such rod-like aggregates showing the changes in the size and configuration of each group with gradually increasing intensities of the attractive force. The arrangement adopted by the units of any one group is, as before, one where attractive and repellent forces are in equilibrium. The conditions naturally become very complex when the number of aggregates exceeds a few, since the attractive force in reality acts separately on each element of each aggregate, and each element is also subject to repulsion by all the other elements. For simplification we may consider all the attractions as resolved in the case of each aggregate into a single resultant acting on one centrally situated point or centre of attraction analogous to the centre of gravity. The aggregates may then be considered in the same manner as if they were single magnets; their central points will thus tend to be brought into the same relative positions as the units in corresponding groups of single magnets, viz., at the angles of an equilateral triangle with three aggregates, angles of a square with four, etc. This disposition will,

however, be subject to disturbance, especially when the aggregates are close together, since the repelling forces, with repellent units of this shape, will not be uniformly distributed. These repulsions will determine the directions of the long axes of the aggregates, and the above-described tendency to parallelism thus resulting will in particular have a very definite influence on the relative positions of the central points. It will be evident on inspection of the figures that

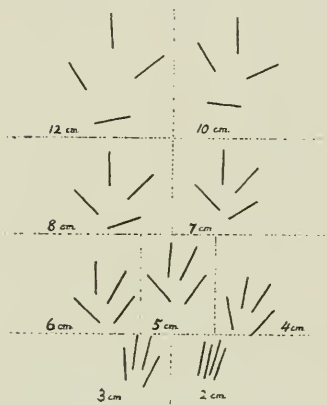


FIGURE 5.—Another grouping of four rods.

as the attractive force increases the figure made by the central points of the rods undergoes considerable change, and that it may be greatly modified, especially when the attractive force is strong, by the mutual action of adjacent rods. The centres of the aggregates become more and more displaced from the theoretical positions where they are disposed similarly to the single floating magnets. This is seen clearly in the series of figures with four rods (Fig. 4); as the four approach parallelism the mutual repulsion between the rods in the direction perpendicular to their length prevents the approximation of their centres, and so tends to disturb the square arrangement; the centres of the aggregates thus occupy approximately the corners of a parallelogram with its long axis perpendicular to the long axes of the rods. A similar distortion of the symmetrical arrangement is seen in the series with five, six, and a greater number of rods as soon as the tendency to a parallel disposition of adjacent rods becomes at all pronounced. In general the explanation of this is simply as follows: whenever several rods in such a system are parallel or approaching parallelism, any displacement of the rods along the general direction of their long axes meets with less resistance from the repulsion of adjacent rods than an equal displacement perpendicular to their long axes; hence the figure made by the centres of the individual rods tends, we may say, to become flattened in the direction indicated by the prevailing direction of the parallel rods (*i. e.*, its short axis lies in this direction). This condition furthers the tendency to a parallelism of adjacent rods so that eventually even so many as six rods may adopt positions side by side and almost parallel.

This we see in Fig. 7. With a larger number of rods individual groups of three or four may form parallel arrangements, and these groups may themselves adopt positions at various angles to one another (Figs. 8, 9).

**Further peculiarities of arrangement.**—Several other features in the behavior of these aggregates should be noted. In Fig. 3 with three

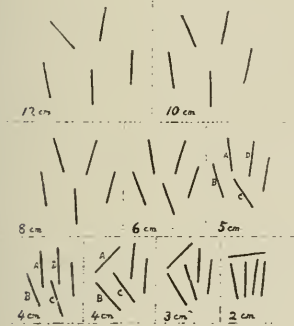


FIGURE 6. — Grouping of five rods. At 4 cm. the configuration undergoes a sudden change. *C* moves in between *A* and *D*; then *A* turns in a clockwise direction as represented, and *B* and *C* shift in the reverse direction. The final configuration of the group is thus determined.

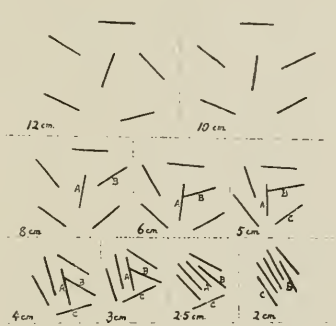


FIGURE 7. — Grouping of six rods with gradually increasing attraction.

rods the 15 cm. configuration is preserved until the attractive pole is 5 cm. from the rods. On slightly increasing the attractive force this configuration ceases to be stable, and one rod shifts into a position midway between the other two. The extremities of the three then diverge slightly, but on increasing the attractive force to 2 cm. they become almost perfectly parallel.

With four rods (Fig. 4) a similar shift of position is seen on increasing the attractive force from 4.5 cm. to 3 cm. Rod *A* shifts into a position alongside *B*. Another series with four rods is represented in Fig. 5. Here the rods, at first strongly divergent, approximate more and more closely to parallelism as the attractive force is increased.

The configurations with five (Fig. 6), six (Fig. 7), and eight rods (Fig. 8) show the same tendency to increasing parallelism with closer mutual proximity of rods. A distinct bilateral symmetry in the

disposition of the elements is noticeable when the attractive force is weak; then the central points are disposed as in the case of single magnets:—with five rods, at the angles of a pentagon; with six, one central and five equally distributed peripheral; with eight, two central and six peripheral. The symmetry of arrangement is dis-

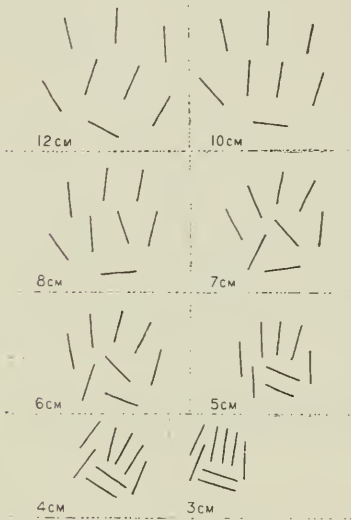


FIGURE 8.—Grouping of eight rods with gradually increasing attraction.

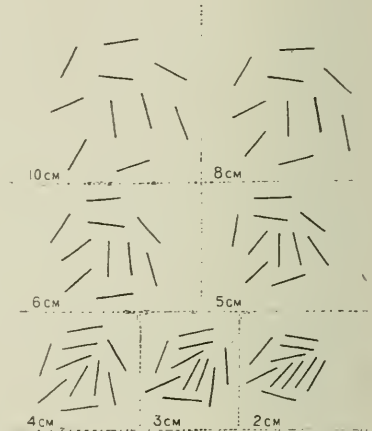


FIGURE 9.—Grouping of ten rods with gradually increasing attraction.

turbed when the attractive force is increased, and the rods begin to dispose themselves in parallel groups.

It may also be noted that if the attractive force becomes sufficiently great, mechanical interference of adjacent rods may result; this is seen, for instance, in Fig. 7, where rods *A* and *B* come in contact when the attractive pole is 6 cm. distant; the shift of position that *B* undergoes later is partly determined by this. Similar conditions probably occur also in the cell.

The group of ten rods (Fig. 9) shows with a weak attractive force the typical arrangement for ten magnets—three central and seven peripheral; and the progressive formation of parallel groups as the attractive force increases is seen in the series of figures.

**Additional conditions determining grouping in cells.**—Before applying the above principles to the phenomena actually observed in cells<sup>1</sup>

<sup>1</sup> Parallel arrangements in groups of rod-shaped chromosomes are common in cells. The following figures will illustrate: In the germ cells of *Ascaris* such

the question must be considered: in what respects can the conditions controlling the behavior of the above models be regarded as truly representing the conditions prevailing in cells, and in what respects are they essentially different? In one respect there is a noteworthy difference: in the cell the chromatic filaments are, except apparently in the equatorial plate stage, free to move in all three dimensions of space, while the models are confined in their movements to the two dimensions of the water-surface. It might be held that the conditions prevailing in the three-dimensional system will differ in no respect from the above except in degree of complexity: that there will, in other words, merely be an increase in the number of stable arrangements possible to a given number of mutually repellent aggregates, but otherwise the conditions and characteristics of the arrangements will be the same. There is, however, one objection to this point of view: although the two filaments floating on a water-surface necessarily occupy one plane, this is no longer true when they are free to move in a third dimension. Under this last condition a parallel position under the action of an attractive force would not be one of stable equilibrium, for the reason that movement in a plane perpendicular to that of parallelism is also possible. The attractive force will, as before, bring the centres of the two aggregates within a certain distance apart, but their mutual repulsions, if opposed by nothing but the attractive force, will tend to place their long axes not parallel but at right angles to one another, as if rod *A* (Fig. 2) were to rotate on its central point in a plane perpendicular to the paper. There would be nothing to prevent this, since movement in such a plane, due to mutual repulsion, is not opposed by the attractive force which acts uniformly in all directions toward the centre. The filaments would, therefore, tend to cross one another at right angles when equi-

arrangements are often seen. Cf. *e.g.*, CARNOY and LEBRUN, *La cellule*, 1897-1898, xiii, p. 63, Plate 1, Fig. 39, etc.; also figures in other papers on *Ascaris* cited in footnote on page 52. See also BÖHMIG: *Zeitschrift für wissenschaftliche Zoologie*, 1891, li, p. 167, Plate 15, Figs. 4-8 (germ-cells of *Plagiotoma*), Plate 16, Figs. 6 and 9 (parallel chromatin rods within the nucleus); HAECKER: *Jenaische Zeitschrift*, 1902, xxx, p. 297, Plate 18, Figs. 18, 19, 20 (typical grouping of rod and loop-shaped chromosomes in germ-cells of *Diaptomus*); Plate 19, Figs. 31, 32 (egg of *Cyclops*); GRIFFIN: *Journal of morphology*, 1899, xv, p. 583; Plate 31, Fig. 16, shows parallel rods in polar bodies of *Thalassema*: BRESSLAU: *Zeitschrift für wissenschaftliche Zoologie*, 1904, lxxvi, p. 213, Plate 14, Figs. 5-10, shows rod-shaped chromosomes with parallel disposition in egg of *Mesostomum*; see also Plate 15, Figs. 19 and 20. Numerous other examples can be found in the literature.



librium was reached, instead of lying parallel. The rule of parallelism, in fact, can apply only to filaments that are compelled by outside conditions to lie in the same or almost in the same plane. That this, however, will be the case for many of the filaments in a three-dimensional system can readily be seen. If we consider two parallel filaments in any such system containing a large number of filaments, so that other repellent filaments occupy adjoining planes, such a movement as the above (leading to crossing) would be resisted by the repulsion of the filaments in adjoining planes. The tendency will thus be, on the whole, for filaments occupying one plane to retain their position in this; and portions of adjacent filaments, whether in the same or in different planes, will tend, as above, to be equidistant so as to balance one another's repulsions. There will thus be a parallelism of arrangement in the case of those adjacent filaments that occupy the same plane.<sup>1</sup> Filaments occupying different planes may, however, cross one another, and this seems to be the case in cells.

#### SPIREME-ARRANGEMENT IN CELLS IS DUE TO PARALLELISM OF ADJACENT FILAMENTS.

With the above limitations it appears that the behavior of the magnetic models will agree with that of mutually repellent filaments in cells. In the spireme stage, for instance, we observe that as the chromatic filaments shorten and become more deeply staining, a tendency toward parallel and approximately equidistant disposition of adjacent filaments becomes evident.<sup>2</sup> Apparently the mutually repellent chromatic particles adopt positions where their mutual repulsions equilibrate one another, and this, if the filaments are similar in their electrical properties, will be when those occupying one plane are equidistant as well as parallel. The result is the production of the usual coiled or roughly spiral figure characterizing this stage of the process.<sup>3</sup>

<sup>1</sup> Or the same curved surface, since the above considerations must also apply to other regular surfaces than the plane (*e. g.*, spherical).

<sup>2</sup> See the section on artificial spireme-formation, p. 72.

<sup>3</sup> Figures of spiremes with filaments showing this typical parallel arrangement are so numerous in the literature that only a few typical examples can be given here. Any one can convince himself by examination of the morphological archives that parallelism of adjacent filaments is the essential feature on which spireme-formation depends. In Protozoa, spireme-like figures are common during cell-

## ARRANGEMENTS OF LOOPED CHROMOSOME-MODELS.

The looped or U-shaped form is so usual with chromosomes (probably because of their frequent formation by segmentation of a looped spireme) that it seems desirable to examine with some care the arrangements shown by groups of U-shaped aggregates under the above conditions. The disposition of U-shaped chromosomes in the equatorial plates of dividing cells shows great variations. At times they appear to be disposed about the periphery of the equatorial plate (usually with their extremities directed outward) the central space being clear. At other times they are distributed uniformly over the entire equatorial plate area.<sup>1</sup> It will be seen that

division. Cf. the figures of micronucleus and macronucleus of Infusoria by R. HERTWIG, reproduced in GURWITSCH'S *Morphologie und Biologie der Zelle*, p. 258, where a striking parallelism of adjacent filaments is shown (see also WILSON'S *The Cell*, etc., 2d ed., p. 89); an equally regular parallelism is shown by the chromatic filaments of *Noctiluca*, according to CALKINS (*Journal of morphology*, 1899, xv, p. 711, Figs. 16, 17, 18, 38); see also BRAUER: *Archiv für mikroskopische Anatomie*, 1894, lviii, p. 189, Plate 11, Figs. 44, 45 (nuclei in *Actinosphaerium*). The following figures illustrate typical nuclear spiremes in Metazoa: HERMANN: *Archiv für mikroskopische Anatomie*, 1891, xxxvii, p. 569, Plate 31, Fig. 13 (spermatocyte of *Proteus*); VOM RATH: *Ibid.*, 1895, xlvi, p. 168, Plate 7, Fig. 23; MEVES: *Ibid.*, 1902, lxi, p. 1, Plate 1, Figs. 17, 18, 19, etc.; VAN DER STRICHT: *Archives de biologie*, 1891, xi, p. 19, Plate 1 (typical spiremes in embryonic liver-cells); DRÜNER: *Jenaische Zeitschrift*, 1895, xxii, p. 271, Plate 4, Figs. 39, 40, etc. (typical spiremes in germ-cells of Salamander), etc., etc.

<sup>1</sup> The ring-shaped disposition is wide-spread, from Protozoa (cf. KEUTEN on cell-division in *Euglena*, *Zeitschrift für wissenschaftliche Zoologie*, 1895, lx, p. 215; Plate 11, Figs. 12, 13), to Vertebrata. Good instances are seen in the germ-cells of Amphibia. Cf. FLEMMING: *Archiv für mikroskopische Anatomie*, 1887, xxix, p. 389, Plate 24, Figs. 24, 28, Plate 25, Figs. 41, 44, etc. (Salamander); DRÜNER: *Loc. cit.*, Figs. 29, 49, etc. (Salamander); BRAUS: *Jenaische Zeitschrift*, 1895, xxii, p. 443, Plate 15, Fig. 20 (egg of Triton); CARNOY and LEBRUN, *La cellule*, 1899, xvi, p. 303, Plate 12, Figs. 118A, 119A, etc. (egg of Triton); MCGREGOR: *Journal of morphology*, 1899, xv, supplement, p. 57 Plate 4, Fig. 16, etc. (germ-cells of Amphiuma).

Curved or looped chromosomes distributed uniformly over the area of the plate also occur. Cf. HAECKER: *Jenaische Zeitschrift*, 1902, xxx, p. 297, Plate 18, Figs. 18, 19, 20, 22, showing division-figures in germ-cells of *Diaptomus* with very regular grouping of small rod and loop-shaped chromosomes; C. BONNEVIE, *Ibid.*, 1901, xxix, p. 275 (egg of *Ascaris lumbricoides*); Fig. 9, Plate 16, shows an equatorial plate with a large number of short, curved rods separated by regular intervals; Fig. 13 shows rounded chromosomes at equal intervals; R. DE SINÉTY: *La cellule*, 1901, xix, p. 119; Plate 2, Figs. 46, 57, show a similar arrangement of



these two types of configuration are similar in their conditions of formation, the difference being due to the existence of a greater polar repellent action in the ring-shaped type of plate (*cf.* pp. 75 *et seq.*).

**General character of arrangements.**—The following figures (10–17) represent accurately arrangements obtained with varying numbers of U-shaped chromosome-models. Configurations with two, three, four, five, six, eight, ten, and twelve of these aggregates are shown.



FIGURE 10.—Arrangements shown by two horseshoe-shaped aggregates. Each loop measures 30 mm. along curve; positions of magnetized needles shown by small circles. All loops in Figs. 10–16 are of similar size and construction. *A*, *B*, *C*, attractive pole 45 mm. above water surface; in *D* considerably nearer.

Fig. 10 represents four configurations with two models. Each model is composed of nine equidistant magnetized needles arranged as represented. Three of the configurations, *A*, *B*, and *C*, represent three possible and stable groupings with a given strength of attractive force (attractive pole 45 mm. above the water-surface). Of these three *A* is the most stable; if the long axis of one chromosome, leaving the other undisturbed, is turned through an angle of  $90^\circ$ , the chromosome always swings back into the original position when released; if turned through a greater angle (as  $120^\circ$ ), configuration *B* may be adopted and will persist. This configuration is also stable within a certain range, though less so than *A*.<sup>1</sup> Of the two chromosomes in *B*, one (that with the concavity outward) can be turned through a large angle

before reaching a point at which it swings into configuration *C*, which is the least stable of the three. A relatively slight disturbance, *i. e.*, turning either chromosome in *C* through a small angle, will result in a return to configuration *B*. If, while the two models remain in the *C*-arrangement, the attractive force is increased, a more stable arrangement is reached, *D*, in which an ex-rod-like or looped chromosomes. See also N. M. STEVENS, *Archiv für Entwicklungsmechanik*, 1902, xv, p. 421; Plate 13, Fig. 15, shows a spindle in the egg of *Echinus* with a large number of short curved chromosomes; these tend to be disposed in such a manner that the curves are approximately parallel and mutual contact is avoided. The chromosomes are in an oval group (like an oblate ellipsoid) with its long axis perpendicular to the spindle-axis; the polar repellent force appears insufficient to bring them all into one plane.

<sup>1</sup> In *A* the attractive centres of the two aggregates (points which lie a little within the central point of each curve) are closer together than in *B*; work must therefore be done on the system to change configuration *A* into configuration *B*. The former is hence more stable.

tremity of one model is nearly midway between the extremities of the other. Under these conditions changing the direction of either axis will bring two pairs of mutually repellent extremities nearer one another, and the original condition will tend to be restored unless the displacement exceeds the critical value beyond which arrangement *B* is adopted. The tendency for the arms of loop-shaped chromosome models to interlock in this manner is very characteristic, and is frequently seen in groups with a large number of chromosomes.<sup>1</sup> (Cf. Fig. 17 with twelve chromosomes.)

The various configurations which a given number of loop-shaped chromosomes may adopt have thus varying degrees of stability. We may say that in a given cell the chances favor the adoption of the most stable configuration.

This is illustrated with three chromosome-models. Configuration *A* (Fig. 11), with all concavities outward, represents the most stable arrangement of three such loops. When the models are placed at random on the surface of the water and allowed to collect at the centre, this configuration in fact usually appears. By shifting the position of one chromosome in the group, configuration *B* may be adopted, which is also stable. A second chromosome may also be turned with extremities inward, giving configuration *C*. But attempts to place all three with extremities inward are unsuccessful; this configuration is unstable, and the system invariably reverts to the *C*-configuration.



FIGURE 11.— Three configurations of three loops. Attractive pole 45 mm. distant.



FIGURE 12.— Three configurations of four loops. Attractive pole 45 cm. distant.

stable,<sup>2</sup> *i. e.*, the central points of the four aggregates are then closest together, and work must be done on the system to pro-

<sup>1</sup> This also occurs in cells. See *e. g.*, CARNOY and LEBRUN, *La cellule*, 1898, xiii, Plate 2, Figs. 3, 22.

<sup>2</sup> Equatorial plates with four loop-shaped chromosomes frequently show this configuration; it is often seen in the division figures of the germ-cells of *Ascaris megalocephala bivalens*; see *e. g.*, NUSSBAUM: *Archiv für mikroskopische Anatomie*, 1902, lix, p. 647, Plate 32, Fig. 27; ED. V. BENEDEN, *Archives de biologie*,

Similarly with four chromosomes (Fig. 12), configuration *A* seems the most

duce any other configuration. Single chromosomes may, as in the preceding case, be shifted into other positions; configurations *B* and *C* are thus produced. But when a third chromosome in *C* is turned with extremities inward, the resulting configuration is found to be unstable, one or other of the inwardly projecting arms is repelled outward, and configuration *C* is resumed.

With five chromosomes (Fig. 13) conditions are more complex. Here, while an arrangement with all concavities outward is possible

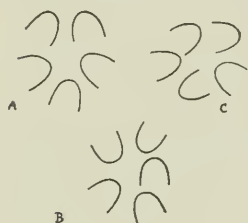


FIGURE 13.—Three random configurations of five loops. Attractive pole 45 mm. distant.

and stable, it seems not to be the one most likely to be adopted. An arrangement with one or more pairs of extremities directed inward apparently brings the central points closer together, and is, as a rule, adopted in random arrangements. Thus *A* and *B* are two such arrangements; four chromosomes first reached the centre, forming configuration *A* of Fig. 12, and the fifth then pushed its way between the others with the result represented. It is, perhaps, noteworthy that the arrangement characteristic of five single magnets — one central,

and four at the corners of a square — while shown by the loops when the attractive force is small and the loops are far apart, is disturbed when the force is increased so as to bring the loops close

1883, iv, Plate 19, Fig. 14; BOVERI: *Jenaische Zeitschrift*, 1888, xv, p. 685, Plate 2, Fig. 44 b, etc. See also KORSCHULT's figures of the germ-cells of *Ophryotrocha puerilis*: *Zeitschrift für wissenschaftliche Zoologie*, 1895, lx, p. 543, Plate 28, Figs. 2 B, 5 C and D, 21, 30. Fig. 5 E shows eight loops in one plane arranged very typically to avoid contact, etc.

The chromosomes in the germ-cells of *Ascaris* are long, slender, and apparently flexible filaments, and adjacent chromosomes often exhibit a striking degree of adaptation to one another's form, bends in one filament fitting into corresponding bends or loops in adjacent filaments, etc. Cf. *e.g.*, Figs. 60, 61, 89 b (with six filaments), and 90 (with five filaments) in above paper of BOVERI. Figures in other papers describing oögenesis or spermatogenesis in *Ascaris* afford numerous instances of the same. See CARNOY and LEBRUN, *La cellule*, 1897, xiii, p. 63, Plate 1, Fig. 39, Plate 2, Figs. 4, 16, 18, 19, 22; SALA: *Archiv für mikroskopische Anatomie*, 1895, xlv, p. 422, Plates 28 and 29, Figs. 77, 78, etc.; POLJAKOFF, *Ibid.*, 1901, lvii, p. 9, Plate 3, Figs. 118, etc.; TRETJAKOFF, *Ibid.*, 1905, lxxv, p. 358, Plate 21, Fig. 16 (spermatogenesis); HERLA: *Archives de biologie*, 1895, xiii, p. 423, Plate 17, Figs. 54, 56, etc. See also numerous figures in BOVERI's *Zellenstudien in Jenaische Zeitschrift*. Compare these figures with those given below (pp. 74-76), showing adaptation of form of artificial flexible repellent filaments to one another.

together; then the central one is repelled to the periphery and one or other of the remaining configurations (*e. g.*, *B*) is adopted. This behavior is due, no doubt, to the asymmetrical position of the central point in each aggregate, so that when the peripheral aggregates approach the central one, the latter is asymmetrically situated with reference to their repulsion and is repelled toward the periphery.

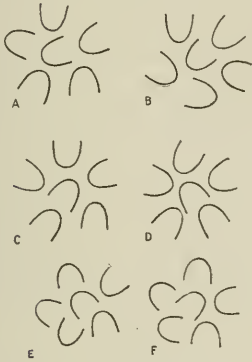


FIGURE 14.— Six configurations of six loops. Attractive pole 45 mm. distant.

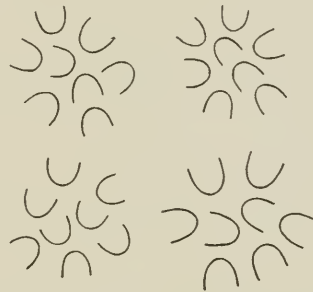


FIGURE 15.— Four configurations of eight loops. Attractive pole 45 mm. distant.

With a larger number of loops this is not so likely to occur, and in fact with six loops (Fig. 14) and the same attractive force as with five the invariable arrangement is one central and five variously disposed peripheral. Attempts to place the six loops in a ring with concavities outward are unsuccessful, one loop invariably shifting to a central position.<sup>1</sup>

In random arrangements of six loops (Fig. 14, *A, B, C*) the majority of the peripheral loops have the concavities outward. It is, however, possible to obtain stable configurations with three or even four concavities directed inward (*E, F*).

Figs. 15, 16, 17 show various stable arrangements with eight, ten, and twelve loops.

**Disposition of extremities of loops.**— One noticeable peculiarity which explains certain features of the configurations is a tendency for the extremities of the arms to be so arranged as to be equidistant from the nearest points of adjacent aggregates. This is so uniform

<sup>1</sup> *I. e.*, if the loops are of the above shape; if the arms were longer, a ring-arrangement might possibly be formed.

as to attract the attention at once; quite frequently an extremity of one arm is found to take a position midway between the two arms of another loop, as in Fig. 10, *D*. The general explanation of this tendency is that each chromosome being free to rotate about an axis perpendicular to the water-surface takes a position where its extremities are equally repelled in opposite directions by adjacent aggregates, the nearest points of which naturally have the greatest influence.

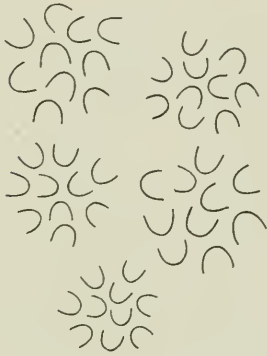


FIGURE 16.— Five configurations of ten loops. Attractive pole 45 mm. distant.

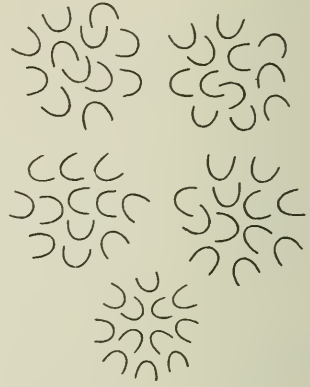


FIGURE 17.— Five configurations of twelve loops. Attractive pole 45 mm. distant.

The result of such action will be that the extremities, when equilibrium is established, will tend to be equidistant from any two points that repel them with equal force in opposite directions. This principle is essentially the same as that which we saw to determine the parallelism of adjacent straight filaments.

Another noticeable feature is the tendency of the peripheral chromosomes to lie with extremities directed outward. This is by no means invariable, but in most random groupings it will be found that the majority of the peripheral loops are so oriented. Configurations with *all* in this position are stable in every one of the above instances, while it is never possible to form groupings with all the peripheral extremities directed inwardly. The general explanation seems to be that the centre of attraction in each loop lies near the central point of the curve, and that when these central points are disposed in positions of equilibrium there may still remain a tendency for each aggregate to rotate on the axis passing through its centre, and for its more readily movable portions to be repelled outwardly by the



repellent action of the system as a whole. These portions are necessarily the extremities of the arms, which hence have a tendency to be directed outwardly. This orientation is very characteristic of looped chromosomes in cells — especially of the chromosomes in ring-shaped equatorial plates.

It will be observed that with eight, ten, and twelve aggregates the loops, considered as units, dispose themselves in a manner similar to that shown by the corresponding groups of single magnets. The precise orientation (*i. e.*, the direction of the axis of symmetry) of each loop is determined by the disposition and orientation of its neighbors, which in turn are determined — collectively speaking — by chance; <sup>1</sup> hence a system of twelve chromosomes may have a very large number of possible configurations. The number of these increases rapidly with any further increase in the number of units composing the system.

Summarizing the above, we may say: rigid curved aggregates (such as many chromosomes actually are) form equatorial plate configurations whose distinctive characters are determined largely by the disposition of the extremities of the loops, which tend to take positions equidistant from the nearest points of neighboring chromosomes, and to be directed outwardly at the periphery of the plate.

**Ring-arrangements of chromosomes.** — Configurations with a large number of looped chromosomes arranged in a ring about a central clear area cannot be produced in the above manner. Since plates of this configuration appear frequently to exist in cells, we can only conclude that the conditions of their formation are of a more complex kind than the above simple arrangement can simulate. One condition appears to be an impenetrability of the axial portion of the spindle by the chromosomes. This may be conceived as due to any one of several causes. The “central spindle” area described by cytologists may be of such physical consistency as to prevent movement of chromosomes in that direction; the configuration of such plates would then be easily explained. Or again, this region may possess the same properties as the astral areas which appear to repel the chromosomes (see below, pp. 77 *et seq.*); a ring-shaped disposition would then also result. The true explanation appears to be that the arrange-

<sup>1</sup> That is: by causes having no relation to the characteristics of the system as an assemblage of mutually repellent structures under the action of an attractive force, but dependent on varying external conditions — as the position in which the loops happen to be when they are placed on the surface of the water, etc.

ment in a plane is due—as will be shown more fully below—to a repulsion from the astral centres, and that a ring-shaped arrangement results whenever the polar repulsion is sufficient to prevent the approach of the chromosomes to the polar axis, where the force of repulsion would naturally be strongest. Hence, while they aggregate in a single plane, they remain at some distance from the central area, which is left clear. The ring-shaped arrangement, in fact, is in itself strong evidence in favor of the view that the astral centres exert a repellent influence on the chromosomes.<sup>1</sup>

#### FORMATION OF ARTIFICIAL SPIREMES.

A general tendency to parallelism of adjacent filaments, conditional on equidistance of equally repelling portions, we saw to be the essential condition of spireme-formation. The methods of formation of artificial spiremes may now be described and some examples given.

In spireme-formation we have to deal apparently with more or less flexible mutually repellent filaments. Artificial filaments having these properties are easily made by the simple process of stringing a series of similarly oriented magnetized floating needles at equal intervals along a flexible thread. In my own experiments I have used silk filaments; the degree of flexibility of these has a marked influence on the configuration of the spiremes, for if the filament is relatively inflexible, conditions approximate to those considered above, where the form of the aggregate is not appreciably altered by the attractions and repulsions to which it is exposed. An imperfectly flexible filament will tend to retain its form, and the final configuration of the system will be determined not only by the attractive and repellent forces between the aggregates, but also by the degree of their resistance to change of form. In the cell the spireme filaments no doubt exhibit all degrees of flexibility; we may assume that this property progressively diminishes as the spireme condenses and approaches the chromosome condition. For theoretical purposes, however, it seems of advantage to consider the filaments first of all as opposing a negligible resistance to change of form while retaining constant length, *i. e.*, as perfectly flexible.

**Spiremes formed from a single flexible filament.**—A single flexible filament composed of a series of mutually repellent magnetized needles floated on the surface of water tends to be drawn out by the mutual

<sup>1</sup> See below, pp. 78, 79.



repulsion of its units into a straight line, since the units strive to become separated as widely as possible. The degree of approximation to this straight form depends on the degree of flexibility of the filament and on the force with which its adjacent portions repel one another. With magnets set close together along a very thin silk thread, the filament becomes almost straight. This fact in itself is interesting, and may have some bearing on such questions as the mode of formation of straight filamentous structures like cilia. Such a filament is in fact in a condition of expansive tension, and therefore tends to increase its length.

Now consider the case of such an elongated filament confined within a circular area of diameter several times smaller than the length of the filament. It will naturally be thrown into some kind of a serpentine or coiled form; its adjacent portions will

however resist the tendency to approach one another, and a condition of equilibrium with adjacent portions approximately equidistant from one another will tend to result. This confinement within a limited area may be due, as in the case of the cell, partly to enclosure by a membrane and partly to the action of a centrally attractive force. The conditions may be simulated by floating an artificial filament of the above-described structure on a water-surface and then placing over it an attractive magnetic pole which draws its constituent portions together within a limited area. The portions of the coil then dispose themselves according to their mutual repulsions. Fig. 18 shows the exact configuration adopted in three separate experiments by a filament 45 cm. in length with 89 needles. The similarity between the three is at once evident, and is seen to be conditional on a parallelism of adjacent portions of the filament. The various curves of the different portions are in fact adjusted to one another, and fit into one another in a manner that suggests conscious adaptation or design. If the figure were in three dimensions, instead of being confined by

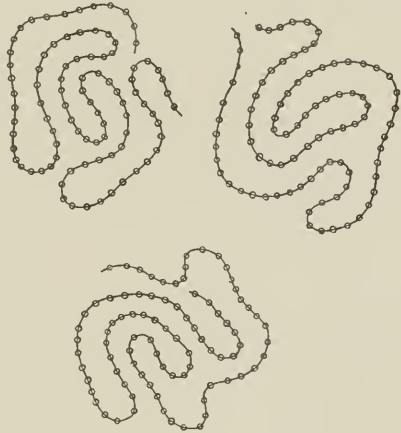


FIGURE 18.— Three spireme configurations from a single slender silk filament 45 cm. long with 89 magnetized needles. Attractive pole 40 mm. distant.

the conditions of its formation to two, we should have a close simulation of the formation of a spireme by a chromatic filament in a cell.

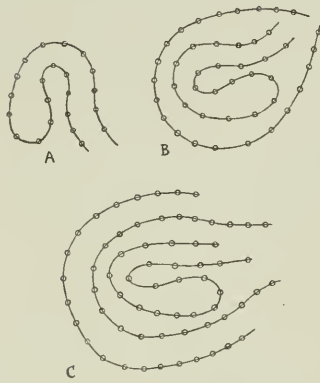


FIGURE 19. — Configurations shown by one, two, and three short filaments, each 185 mm. long and with 21 or 22 needles. Attractive pole 30 mm. distant in *A* and *B* and 40 mm. in *C*.

therefore that in a spireme containing many filaments those situated centrally would be the closest together. The same is seen in the artificial spiremes with six separate filaments (Fig. 22). According to this a spireme-formation should in general be looser in structure toward its periphery than at its centre, if its structure depends in the above-described manner on the mutual repulsion of its filaments.

**Spiremes formed from several filaments.** — Figs. 19 to 22 show artificial spiremes made from varying numbers of filaments, one to six. Each filament consists of 21 or 22 needles strung along a silk thread somewhat less flexible than the one used in the previous experiment. The effect of a greater stiffness of the threads is seen in the slower curvature of the bends when loops are formed, and in the less perfect adaptation of the form of one filament to that of its immediate neighbor. In Fig. 19 with one,<sup>1</sup> two, and three filaments, the typical

It is noticeable that toward the centre of the figure adjacent portions of the filament are closer together than at the periphery. The explanation of this is simply that the central portions are subject to a centrally directed repulsion from the peripheral portions of the filament (in addition to the centrally directed attraction of the large magnet); this centrally acting force in some degree compensates the mutual repulsion of the central portions of the thread, whereas the more peripherally any filament is situated, the less is it subject to a centrally directed repulsion, and at the outside such action is entirely absent.

We should expect

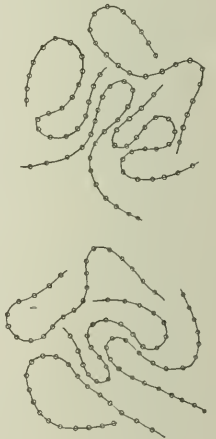


FIGURE 20. — Artificial spireme with four filaments like those of Fig. 20. Attractive pole 45 mm. distant.

<sup>1</sup> The form of this loop is almost identical with that of the single chromatic thread in the germ-cells of *Thalassema*, according to GRIFFIN, *Journal of mor-*

parallelism, is seen. In Fig. 20 a somewhat complicated system of loops is formed, but the general disposition follows the same rules as before, although here the comparative inflexibility of the thread prevents close approximation of the portions of a filament immediately adjoining the bend; this gives a tendency to the formation of wide loops and interferes with the tendency to parallelism. Fig. 22 shows three arrangements with six such filaments, and Fig. 23 shows three

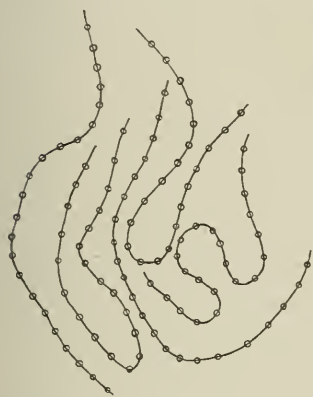


FIGURE 21. — Artificial spireme with five filaments like those of Fig. 20. Attractive pole 45 mm. distant.

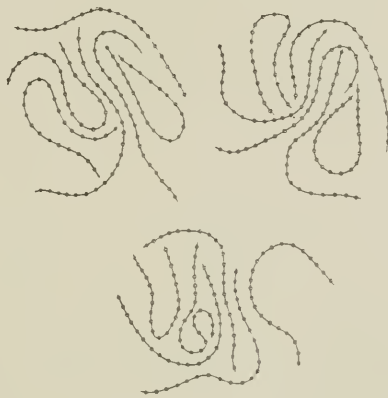


FIGURE 22. — Three artificial spireme figures with six filaments. Attractive pole 45 mm. distant.

arrangements of twelve short flexible filaments, each with seven magnets; the frequent parallelism of adjacent filaments and the adaptation of their forms to one another are shown clearly; this last occasionally results in the adoption of a looped or ring-like form.

#### FORMATION OF THE EQUATORIAL PLATE.

**Nature of equatorial plate arrangement in cells.**— The constant characteristic of the equatorial plate-arrangement is simply: that the chromatin-aggregates, instead of being distributed as before in a manner which as a rule shows no definite relation to any axis of the cell, become disposed side by side in a single plane definitely oriented with reference to the two polar radiating areas which have meanwhile appeared in the cytoplasm. These areas in some manner influence the phology, 1899, xv, Plate 31, Fig. 2. Parallelism of rod-shaped chromosomes is seen also in Figs. 15, 16.

disposition of the chromosomes so that the latter eventually are brought into a plane perpendicular to the line joining the centres of radiation and precisely midway between the two. This position is evidently one of equilibrium, for it persists until the longitudinal division of the chromosomes has occurred, when the two groups of daughter-chromosomes recede toward opposite poles of the cell.<sup>1</sup>

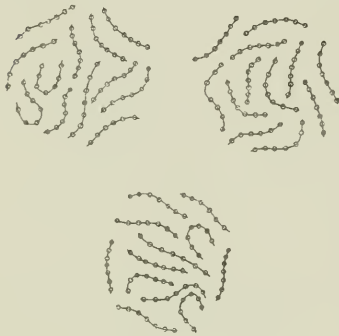


FIGURE 23. — Three figures with short flexible threads each 35 mm. long with 7 needles. Attractive pole 40 mm. distant.

The question is: what are the conditions of equilibrium in the equatorial plate stage? Can it be explained as due to a balance between attractive and repellent forces of a purely electrostatic kind? or must we assume, as the majority of cytologists have done hitherto, that the arrangement is produced by the mechanical action of a system of contractile spindle-fibres? The latter hypothesis appears to have

some basis of fact, since fibre-like formations, apparently inserted into the chromosomes, are usual in cytological preparations of dividing cells. One difficulty here is to decide how far these fibrils exist as such in the cell, and how far they are artificially produced through the coagulative action of the fixing fluids on a substance which, in the living state, is not fibrillar, but merely in a peculiar condition (usually described as some kind of strain) that leads its colloids to form fibrillar aggregates on fixation. Similar artificial fibrillar structures can in fact be produced (as shown by the work of Fischer, Hardy, Bütschli and others<sup>2</sup>) in colloid systems that, previously to fixation, can have no such structure. It is thus possible that the astral fibrillæ do not exist as such in living cells. The very difficulty of understanding how a system of contractile fibrils can be so delicately adjusted as to produce such a striking uniformity of arrangement is in itself an objection to the mechanical theories in their usual

<sup>1</sup> The separation of the two daughter-chromosomes and their passage toward opposite poles is probably due, at least in part, to the strong mutual repulsion between the two closely adjacent products of division.

<sup>2</sup> A. FISCHER: *Fixierung, Färbung und Bau des Protoplasmas*, Jena, 1899; HARDY: *Journal of physiology*, 1899, xxiv, p. 158; BÜTSCHLI: *Verhandlungen der medicin.-natur. Gesellschaft, Heidelberg*, '1892.

form. That is to say, they are not adapted to simplifying our conceptions of the nature of the process.

On the other hand, a combination of attractive and repellent forces may readily be imagined, that will give precisely the typical arrangement, viz.: in a single plane, midway between two poles, and perpendicular to the polar axis. It is necessary to assume (1) that the chromosomes are attracted toward a region midway between the astral centres, and (2) that some condition resists their approach toward the poles, while leaving them free to move in a direction perpendicular to the spindle-axis. Under such conditions they will repel one another outward along this plane and adopt positions within it, where their mutual repulsions are in equilibrium with the centrally attractive force.

What is the nature of this second condition? The fact that the position of equilibrium is midway between the astral centres indicates that these have equal action on the chromosomes, and in opposite directions. If the action is one of attraction, as generally supposed (hence the customary designation, *attraction-sphere*), it is difficult to understand why the chromosomes remain stationary, since such a position, if the attraction is similar to that of other attractive forces, would be one of unstable equilibrium, and the slightest displacement of a chromosome toward either pole would lead to its being drawn toward the latter, unless this were prevented by the action of some automatically compensating mechanism (such as one might suppose the astral fibres to be). On the other hand, many considerations support the supposition that the astral regions repel the chromosomes; such are the facts (1) that the midway position is one of apparently stable equilibrium, (2) that in triastral or tetrastral formations the chromosomes form central groups whose peripheral portions extend outward midway *between* adjacent asters, and lastly, (3) that by a suitable arrangement of one central attractive and two polar repellent magnetic poles, groups of floating magnets can be made to dispose themselves side by side in a row midway between the two repelling poles, and perpendicular to the line joining them — in fact, to exhibit the typical equatorial plate arrangement.

**Artificial simulation of equatorial plate arrangement.** — This arrangement can be produced in the following way. A group of (for example) ten single floating magnets is brought to a central position under the action of an attractive magnetic pole. It is preferable that this attraction be due to a strong bar-magnet, or to a combination



of several of these, suspended at some distance above the surface of the water; under these conditions the attractive force diminishes only gradually as the floating magnets diverge from the central position.

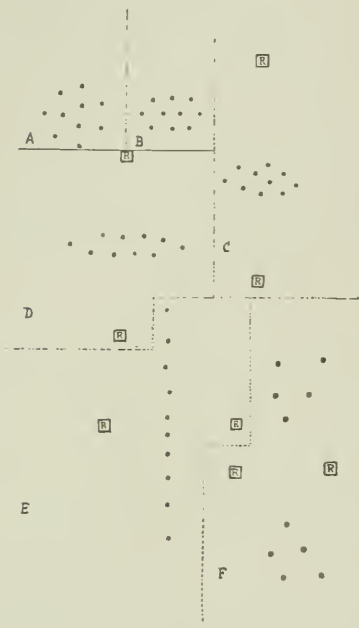


FIGURE 24. — Illustrating formation of plate arrangement. *A*, normal arrangement of 10 floating magnets. *B*, configuration with two repellant poles at considerable distance. *C*, *D*, and *E*, configurations with repellant poles (*R*) in position represented. *F*, configuration with repellant poles still nearer group of magnets (corresponding to a section through a ring-grouping of chromosomes).

This attractive action corresponds to the assumed attraction toward the mid-interastral area. Two weaker repellent poles of equal strength are then brought into equal proximity with the floating group from opposite sides; the result is that the individual magnets are repelled toward the centre and outward along the direction of the resultant formed from the two sets of repelling forces, until the united repulsions are once more balanced by the centrally acting attractive force. A new configuration of equilibrium results with the long axis of the group perpendicular to the line joining the two repellant poles. Fig. 24 *B* and *C* shows the disposition of a group of ten units when the polar repelling forces are slight, *i. e.*, the repellant magnetic poles at some distance from the group. Increasing this somewhat gives configuration *D*, where the units show a regular arrangement in two rows. *R* and *R* indicate the positions of the repellant poles. As the polar repulsion increases, the group spreads out laterally more and more, until at a

certain position of the poles configuration *E* appears with the ten units side by side in a single plane, perpendicular to the line joining the two repellant poles, and precisely midway between them. This corresponds to a section through an equatorial plate of about 75 chromosomes. The configuration is stable; equilibrium depends on a balance between three sets of forces, (1) the central attractive force drawing the chromosomes toward a region midway between the two repellant poles; (2) the repulsion between the individual chromo-

somes; and (3) the repulsion exerted on these latter by the two repellent poles. When equilibrium is reached, the chromosomes are found to be at approximately equal distances apart, each is equidistant from the two repellent poles, and the row is perpendicular to the line joining these. The chromosomes are more strongly repelled outward in the plane perpendicular to the polar axis than in any other direction, because in this plane the repellent action from the two poles is added to that between the individual chromosomes. The two poles compensate one another's tendency to displace a chromosome in any direction except outward along this plane; their united actions thus tend to bring the chromosomes into the median plane, and to repel them outward along it until the repulsion is balanced by the centrally directed attractive force.

The greater the polar repellent forces become, relatively to the attractive, the greater is the tendency for all the chromosomes to shift into the median plane and to adopt side-by-side positions within it. If then the polar repulsions are still further increased, the chromosomes may be completely repelled from the central area, and form two groups on either side of it, and at some distance from the inter-polar axis (Fig. 24 *I'*). This configuration corresponds to a ring-shaped grouping in an equatorial plate with the central space clear and the chromosomes distributed around the periphery of the spindle area. The ring-shaped grouping in cells is then to be regarded as due to the existence of a polar repellent force so strong as to prevent the approach of chromosomes toward the spindle axis, while permitting their collection at some slight distance from it.

It is also evident that if the chromosomes are elongated or loop-shaped, the same conditions that keep individual rounded units in the one plane will also keep the different portions of the single elongated chromosome in the same plane. Hence such chromosomes will at the metaphase lie with their long axes and both their arms in the equatorial plane. They will also tend as the result of conditions considered above (p. 70) to lie with the extremities directed outward.<sup>1</sup>

The above groupings evidently correspond to *sections* through equatorial plates in the plane of the polar axis. It is clear that the

<sup>1</sup> Cf. GURWITSCH'S *Morphologie und Biologie der Zelle* (FISCHER, Jena, 1904), p. 272, Fig. 179, for a figure that illustrates at once the repulsion of chromosomes from an aster, the ring-shaped grouping, and the tendency of the extremities of the loop to be directed outward (oögonium of the guinea-pig).



confinement of the chromosome models to the plane of the water surface is dependent on an inessential and arbitrary condition of this method of experimenting. If the mutually repellent units could move in three dimensions instead of only in two, it is evident that the arrangement corresponding to the row (Fig. 24, *E*) would be a flat circular plate cutting the inter-polar axis at right angles and with its units distributed at nearly uniform intervals over its entire area.

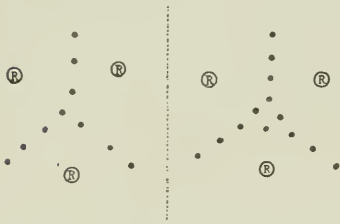


FIGURE 25.—Triradiate arrangement of groups of ten and thirteen single floating magnets. Position of repellent poles shown by *R*. These belong to short bar-magnets each ca. 120 mm. long, inclined downward and inward toward centre of group. Attraction due to two large bar-magnets each 250 mm. long, suspended in vertical position above centre of group.

Corresponding to a single row of ten equidistant units would be a circular equatorial plate of about 75 chromosomes. And corresponding to the grouping of Fig. 24 *F* would be a ring in the same plane.

The above combination of attractive and repellent forces can therefore account for the formation of equatorial plates in cells and for certain of the most characteristic variations in the configuration of these. The uniform mode of distribution and the ring-shaped configuration can be reduced to the same conditions, the difference depending on variations in the strength of the polar repellent forces relatively

to the attractive force, polar repulsion being greater in the ring-shaped formation.

**Artificial simulation of triaster formations.**—Further evidence that the astral areas repel the chromosomes is seen in the characteristic distribution of the chromatic matter in abnormal polyastral mitoses. In triasters the chromatin, at a stage corresponding to the metaphase, is grouped in a triradiate manner with the rays extending outward midway between the centres. Similarly in tetrasters, when cross-like grouping of chromosomes is usual, the arms of the cross extending outward midway between the asters and tending to be perpendicular to the lines joining adjacent pairs of these.<sup>1</sup> Other polyastral figures may

<sup>1</sup> Cf. for typical examples GALEOTTI: Ziegler's Beiträge zur pathologischen Anatomie, 1893, xiv, p. 288, Plate 15, Fig. 2 (triradiate disposition of chromatin); also *Ibid.*, p. 249, Plate 12, Fig. 12 (triradiate) and Fig. 14 (tetrastrate or cross-shaped grouping with asters in interspaces of arms). Cf. also above-cited paper of M. BOVERI, *loc. cit.*, p. 421, Fig. *R*.

show more complicated arrangements. The interesting feature of such formations is that they confirm the view which ascribes a repellent action to the astral centres. The chromosomes in such poly-astral mitoses apparently tend to adopt positions in planes midway

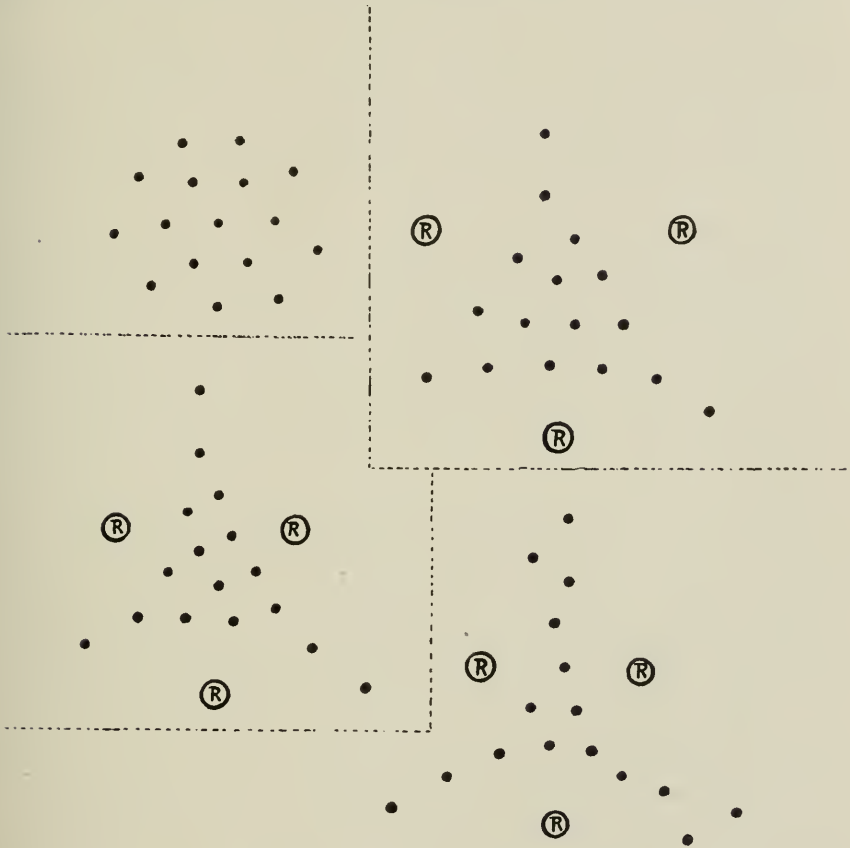


FIGURE 26. — Group of 16 floating magnets showing gradual adoption of triradial configuration as repellent poles are approached to the group.

between adjacent asters; this is to be expected on the foregoing theory, and in fact this disposition can, in simple cases, also be simulated by the above method. Thus Fig. 25 *A* shows a triastral arrangement of ten needles produced by a combination of three similar repellent poles and one strong centrally attracting pole, and Fig. 25 *B* shows thirteen needles in a similar arrangement. In Fig. 26 is given a series of configurations of a similar group of sixteen

needles showing the progressive adoption of the triastral arrangement as the repellent poles are more and more closely approximated to the group; in the last figure the most peripheral chromosomes are not repelled with a force sufficient to keep them in a straight line with the others, but the essential features of this mode of disposition are shown. The determining conditions of such configurations are in principle the same as in the formation of the simple plane equatorial plate, the difference being merely that the chromosomes assume a disposition with reference to more than one pair of repelling poles. The same considerations apply when a larger number of poles are used.

**Nature of astral formations in cells.** — The above experiments show that the arrangement of the chromatic matter in the equatorial plate can be explained on the assumption that the astral areas repel the chromosomes. Is there any other justification for such a view? To what physiological changes actually occurring in the cell can the production of such repellent areas be referred, which, if the repulsions are electrostatic and the chromosomes electro-negative, *must represent negatively charged areas?*

We are ignorant of the precise nature of the changes that lead to the formation of these peculiar radiating areas in the cytoplasm of dividing cells. It seems highly probable, however, that a change in the aggregation-state of the cytoplasmic colloids may be one condition, though this again is to be referred to the production of the localized potential-differences of which aster-formation is merely the visible expression. By producing artificially aggregation-changes in colloidal systems Bütschli and Fischer have, in fact, produced radiating arrangements of particles similar to those seen in cells. The appearance of the "attraction-spheres" and spindle-fibres in cytological preparations also suggests strongly that an aggregation of colloidal particles, in other words, a change in the direction of coagulation, is occurring in these regions. Or the change of aggregation-state may, in many cases, perhaps, be in the reverse direction; thus in living dividing echinoderm-eggs the astral areas have a clear appearance as if of more liquid consistency than the rest of the cytoplasm. Such a change would be produced by negative charges on a colloid system with negatively charged particles. Changes in aggregation-state of the cytoplasmic colloids, in whatever direction it might be, would certainly result from changes of electrical potential in the different regions of the cell. However, the idea that the

definite arrangement of colloid particles forming the characteristic system of radiations is due to a polarization analogous to that of particles in electrical or magnetic fields seems most deserving of consideration; the apparent correspondence of the figure presented by astral radiations and spindle-fibres to the lines of force has long given a certain justification to this view. It meets with various difficulties, however, when attempt is made to apply it in detail; thus it has seemed to some authors necessary to ascribe an opposite polarity to two adjacent astral centres connected by a spindle-like arrangement of fibres since the lines of force between *opposite* electric or magnetic poles alone exhibit this form.<sup>1</sup> Yet the identity in structure and microchemical reactions of such areas proves that they are identical in their physico-chemical characteristics, and hence in the sign of the electrical charges which they probably bear. Another apparent difficulty is that the disposition of the spindle-fibres with reference to the chromosomes, into which they often appear to be inserted, seems to force us, on the hypothesis that spindle-fibres mark out the electrical lines of force, to ascribe opposite electrical polarities to chromatin and astral centres; whereas all the above considerations point to their bearing like and in fact *negative* charges.

On the theory upheld above, that the astral centres are negative areas and that for this very reason the interastral areas must be positive, many of the above difficulties disappear. The spindle-shaped arrangement of the fibres between adjoining centres, whether connected with chromosomes or not, is in accordance with this theory, since each negative area or astral centre in the typical spindle-formation is then connected with the central interastral positive area by the required curved system of lines of force, with the concavities toward the axis. The direction of the spindle-fibres thus appears to correspond with the lines of force, as they should do if they are formed by the polarization of the colloid particles in the electrical field. The attraction of the chromosomes into the central positive area need not necessarily disturb the spindle-arrangement of the fibres, and in fact does not usually appear to do so. In some cases however the different distribution of the "mantle-fibres" and the "central spindle-fibres" may be due to the influence of the chromosomes, which as negatively charged bodies may at times exert a perceptible influence on the direction of the lines of force in the spindle-area.

<sup>1</sup> Cf. RHUMBLER: *Archiv für Entwicklungsmechanik*, 1903, xvi, p. 476.

I shall not discuss these questions further in this place, since decisive facts seem wanting at present. What we need is an explanation of the production of localized electro-negative areas in the protoplasm, for such the astral areas almost undoubtedly are. This is a problem for physiological chemistry; it is obvious that certain chemical changes might lead to the production of such areas, since many if not all chemical actions are, according to the conceptions of the electron theory, dependent on transfer of electrical charges between the interacting molecules, ions, or atoms. It is easy to understand that changes of a certain kind might leave one area deficient in electrons (*i. e.*, positive) and another with a surplus (negative). Many facts indicate that there is produced a strong electrical field within the cell during mitosis; but the exact chemical changes that lead to the production of this field and determine its character can only be ascertained by further experiment.

#### SUMMARY.

1. The disposition and relative positions of many colloid aggregates in the cell, especially the chromosomes and chromatic filaments during mitosis, indicate that mutual electrostatic attractions and repulsions play an important part in determining their position and movements.

2. By the use of mutually repellent groups of floating magnetized needles exposed to the attractive or repellent action of magnetic poles many features in the above arrangements can be simulated, particularly spireme-figures and the arrangement of the chromosomes in the equatorial plates of normal and tripolar mitoses.

3. The conditions necessary for the simulation of equatorial plate formations indicate that the astral centres have a repellent action on the chromosomes, and therefore, since chromosomes are negatively charged aggregates, these areas must also be negatively charged. The corresponding positive charges are apparently situated in the interastral area and toward the surface of the cell.



## A PHARMACOLOGICAL STUDY OF ANESTHETICS AND NARCOTICS.<sup>1</sup>

BY ORVILLE HARRY BROWN.

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THE group of drugs comprised under the head of narcotics and anesthetics has a physico-chemical affinity for fats and fat-like bodies. The cell protoplasm of animal tissues, being rich in lipid bodies, takes up and retains, for a greater or less time, these compounds when they are introduced into the system. Pohl<sup>2</sup> found that the blood of an anesthetized animal gave up the chloroform it contained so slowly that air could be passed through the blood for twenty-four hours without causing all the chloroform to disappear. The richer the cell is in the lipid bodies, the more of the anesthetic it will take up, and the more difficult it will be to free it of the narcotic compound.

Meyer<sup>3</sup> and Overton's<sup>4</sup> hypothesis of the mode of the production of anesthesia by these compounds is based upon the chemical law that where two substances have dissimilar dissolving power for a certain substance, the better solvent gets the greater amount of the substance. From the work of the above investigators it seems that the degree of activity of each of the narcotics is approximately proportional to the physico-chemical affinity between it and a lipid; *i. e.*, the compound with the highest solution tension for fat-like bodies and the lowest solution tension for watery media will be the most active as an anesthetic. The nerve cells, being rich in cholesterin and lecithin bodies, will accumulate a proportionately large amount of

<sup>1</sup> This work was done the past summer while occupying a Carnegie research room of the Marine Biological Laboratory at Woods Hole.

<sup>2</sup> POHL: *Archiv für experimentelle Pathologie und Pharmakologie*, 1890-91, xxviii, p. 239.

<sup>3</sup> MEYER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1901, xlii, p. 109.

<sup>4</sup> OVERTON: *Studien über der Narkose*, Jena, 1901.



a narcotic when it is introduced into the system. The origin of narcosis is undoubtedly the chemical compound in combination with the lipid of the cell. Meyer and Overton's hypothesis holds that the liquefaction of the cell occasioned by this fat-narcotic compound causes a derangement of the normal condition of the cell, and consequently an impairment of its function, which is narcosis. Hans Meyer<sup>1</sup> in a recent lecture at the Johns Hopkins medical school presented the idea that the lipid may belong to the essentially active constituents of the cell. Therefore narcosis is a result of the throwing out of function of the life-centre of the cell by a union of the compound with this centre. But he adds that an objection to the above has been raised that the lipoids may not be connected with the necessary functioning portion of the cell; hence they merely cause an accumulation of the narcotic, which then acts on the true albuminoid life-centre of the cell in proportion to the degree of accumulation.

The functions of the cell, being carried on as far as known by enzymes, are very apt to be profoundly affected by the presence of a chemical substance; it has been shown by many investigators that most chemical substances have a distinct influence upon enzyme activities, if present with the enzymes in sufficient concentration. A few of the recent workers on this problem are mentioned in the bibliography. Neilson<sup>2</sup> and Terry<sup>3</sup> found that narcotics have a marked retarding influence on the splitting of hydrogen dioxide by a watery extract of kidney. Although they tried only a small number of compounds, it seems safe to say that the depression of the catalysis by a narcotic is in proportion to the narcotic power of the compound. It seems reasonable, as suggested by these investigators, that narcotics produce their effect, partially at least, by an inhibiting influence upon the enzymes of the cells.

Meltzer and Auer,<sup>4</sup> in a recent pharmacological investigation of

<sup>1</sup> MEYER: *Science*, 1905, xxii, p. 417.

<sup>2</sup> KUBEL: *Archiv für die gesammte Physiologie*, 1899, lxxvi, p. 276; OPPENHEIMER: *Die Fermente und ihre Wirkungen*, 1900; COLE: *Journal of physiology*, 1903, xxx, p. 202; BREDIG: *Anorganische Fermente*, Leipzig, 1901; BRAUENING: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 80; KASTLE and LOEVENHART: *American chemical journal*, 1903, xxix, pp. 397, 563; MCGUIGAN: *This journal*, 1904, x, p. 44; NEILSON and BROWN: *Ibid.*, 1904, x, pp. 225, 335; *Ibid.*, 1904, xii, p. 374; *Ibid.*, 1905, xiii, p. 427.

<sup>3</sup> NEILSON and TERRY: *This journal*, 1905, xiv, p. 248.

<sup>4</sup> MELTZER and AUER: *This journal*, 1905, xiv, p. 366.

magnesium salts, found that magnesium sulphate in a proper dose will produce anesthesia with complete relaxation of all the voluntary muscles, and abolition of some of the important reflex activities. A large dose of the salt produces anesthesia and general paralysis, and finally death. Magnesium chloride acts like the sulphate.

In an earlier paper the author of this article, in conjunction with Neilson,<sup>1</sup> showed that magnesium chloride inhibited very markedly the splitting of hydrogen peroxide and the hydrolysis of butyric ether by platinum black and extracts of pancreas and kidney. The fact that magnesium chloride is capable of producing anesthesia and inhibiting enzyme action is evidence that there may be some dependence of the latter upon the former.

Nef's<sup>2</sup> bivalent carbon hypothesis is made the basis by A. P. Mathews<sup>3</sup> of a plausible hypothesis for protoplasmic respiration. The bivalent carbon compound of the protoplasm, which may be either simple or complex according to Mathews, decomposes the water of the tissues into its elements. The oxygen combines with the compounds constituting protoplasm, thus oxidizing them. The hydrogen is united with free oxygen or other substances in the tissues, or passes off as gas. Mathews's<sup>4</sup> idea of the action of anesthetics is that they inhibit the action of the bivalent carbon, thereby decreasing the respiration of the cell protoplasm, and this results in the stage known as anesthesia. It seems to the author of this article, however, that it is more likely that the substances producing a narcosis do so, not by an action on any one of the essential processes of the protoplasm, but from the combined influence on all of them. The rôle that the lipoids of the cell play in narcosis may be only that of a solvent or a gatherer for the narcotic, or more, depending upon whether or not the lipid is concerned with the essential living processes of the cell.

The author has, for his object, in the experimental portion of this paper, a comparative study of solutions of a number of the compounds in common use as anesthetics, narcotics, and hypnotics, — the concentration being expressed in terms of the gram-molecule. Several summers ago while at Wood's Hole Dr. Mathews called the attention

<sup>1</sup> NEILSON and BROWN: This journal, 1904, x, p. 225; *Ibid.*, 1904, x, p. 335.

<sup>2</sup> NEF: Liebig's Annalen, 1904, cccxxxv, p. 192 (reference from Biological bulletin, 1905, viii, p. 335).

<sup>3</sup> MATHEWS: Biological bulletin, 1905, viii, p. 331.

<sup>4</sup> MATHEWS: Personal communication.

of the author to the fact that starfish eggs were greatly affected by chloroform, ether, etc. This effect appears to be a partial liquefaction of the protoplasm. It was suggested by him that possibly the power of each member of this group of compounds to liquefy the starfish eggs might be proportional to its narcotic power. Such a piece of work was carried out at that time, but for various reasons was not published. The past summer the work was repeated and extended.

The change produced in the eggs is a profound one. The eggs enlarge, and become lighter in color; the protoplasm becomes less granular, and finally there is a rupture of the envelope at some spot, and the contents flow out. Herman<sup>1</sup> observed a similar change in red blood corpuscles when treated with anesthetics. The process indicates that the contents of the cell have been increased in amount and fluidity. The explanation seems to be that the narcotics are taken up by the fat-like bodies of the egg. The term liquefaction will be used in this paper to represent the above process. Very similar changes were observed by Sollmann,<sup>2</sup> as a result of placing the eggs in anisotonic solutions. The two processes may be the result of a passage of liquid into the cell; water in Sollmann's experiments and chloroform, ether, etc., in the author's. In the former the water entered on account of a higher osmotic pressure on the inside than on the outside of the cell; in the latter the narcotic compound entered the egg from a solution which was hyperisotonic to the egg, on account of the physico-chemical affinity between the compound and lipid of the cell.

An unexpected phenomenon was found. Chloral hydrate in 1 mol. concentration did not cause liquefaction of the eggs. On the contrary, the eggs became denser, more granular, and possibly a little smaller. No liquefaction occurred even after the eggs had been in the solution for several hours. In the more concentrated solutions the results are the same. In a  $\frac{5}{8}$  mol. solution a small per cent of the eggs show some liquefaction. This consists of a narrow area on the border, which becomes lighter in color and shows less granular material. The degree of liquefaction gradually increases in indirect ratio to the concentration of the solution, until a certain dilution causes complete liquefaction of the eggs. A  $\frac{1}{8}$  mol. solution causes liquefaction of about 99 per cent of the eggs in one minute. From this point the

<sup>1</sup> HERMAN: *Archiv für Anatomie, Physiologie, und wesentliche Medicin*, 1866, p. 27.

<sup>2</sup> SOLLMANN: *This journal*, 1904, xii, p. 99.

percentage of eggs liquefied decreases as the concentration grows less. The chloral hydrate solutions were made up with sea water, and it seemed possible that the hyperisotonic solution might be responsible for the results. In order to eliminate this factor, a  $\frac{5}{8}$  mol. solution of chloral hydrate was made in distilled water. This solution

TABLE I.

Compound.	Molecular concentration.		Time.	Per cent of eggs liquefied.	Notes.
	gm.-mol.	gm.-mol.			
Chloral hydrate in sea water	$2\frac{1}{2}$	2.5	hrs. min. 3 0	None	Eggs became denser and darker.
“ “ “ “	1	1	1 30	5	Just a small area on border affected.
“ “ “ “	$\frac{1}{2}$	0.5	1 30	25	Not complete. Area on border extended.
“ “ “ “	$\frac{1}{4}$	0.25	1 30	50	Not complete. Area increasing.
“ “ “ “	$\frac{1}{8}$	0.125	0 1	99	Liquefaction complete.
“ “ “ “	$\frac{7}{80}$	0.0875	0 1	95	
“ “ “ “	$\frac{3}{40}$	0.075	0 1	90	
“ “ “ “	$\frac{1}{20}$	0.05	0 1	10	
Chloral hydrate in distilled water	$\frac{5}{8}$	0.625	1 0	10	Just a small area on border.
“ “ “ “	$\frac{5}{4}$	0.078	0 1	90	

should be approximately isotonic with the sea water. The results with this solution were identical with those obtained by the use of the solution made up in sea water. The distilled water solution on being diluted with sea water sufficiently caused liquefaction of the eggs. Equal concentrations of the two solutions caused the same results upon the eggs. Chloral hydrate, upon standing, undergoes some decomposition, which renders it acid. On the assumption that possibly the acid of the concentrated solutions might counteract the liquefying power of the chloral hydrate, the solution was neutralized. This did not alter the results, however.

In Table I is shown the concentrations of the chloral hydrate solutions used, and the time that the eggs were observed after being put into the solution, and the amount of liquefaction produced by it, in the various concentrations.

Several of the other narcotic compounds were tried in concentrated solutions and were found to act the same. A great many of these

compounds, though, are not sufficiently soluble to get a solution of the necessary concentration.

It is a well-known fact that a concentrated solution of alcohol precipitates and coagulates albumin. Sollmann,<sup>1</sup> in his chapter on the action of the chloroform-alcohol group of drugs, says that these two compounds and many of the others of the group cause a precipitation of albumin. Chloral hydrate solution, the author finds, causes a coagulation on contact with albumin. The explanation of the two different actions of chloral solutions on the eggs of the starfish seems to be that in the concentrated solutions the coagulation of the albumin is a pronounced process, and inhibits the union of the compound and the lipid, while in the dilute solutions this occurs to but a slight degree, and permits the affinity between the fat-like body and the narcotic to be effective. The coagulation in the former case may make the egg envelope less penetrable to the narcotic, and in this manner counteract the affinity between the two substances.

The possibility has been suggested by Professor Lyon<sup>2</sup> that the anesthetics in the dilute solutions may bring about the liquefaction of the eggs, because of a disintegration of some constituent of the protoplasm, caused by the anesthetics. It has been noticed by him that paramœcia, which will normally live in distilled water, when placed in sugar solutions first shrink in size and then swell up and go to pieces. The shrinkage is evidence of the hypertonicity of the solution; the cause of the going to pieces must then be the splitting of some constituent of the protoplasm, which increased the osmotic pressure of the cell.

To compare the power of the chemicals used as narcotics and anesthetics, to liquefy starfish ova, solutions of the compounds were made in sea water. Ten cubic centimetres of a solution were placed in a small salt-cellar. To prepare the eggs for the experiment, all of the surplus sea water in the dish containing the eggs was removed by a pipette, leaving a great mass of eggs surrounded by a small amount of sea water. The eggs had previously been freed of all ovary tissue. Three drops of the eggs were added to the solution in the glass dish, and thoroughly mixed immediately by drawing the solution and eggs up into a medicine dropper and squirting out two or three times. The dish was then placed under the low power of a microscope, and one minute after the eggs had been put into the solution, an estimate

<sup>1</sup> SOLLMANN: SOLLMANN'S Pharmacology.

<sup>2</sup> LYON: Personal communication.



was made of the percentage of the eggs liquefied. The small amount of the sea water introduced with the eggs, the time required for the mixing, and the temperature were practically the same in each test.

The estimation of the percentage of the eggs liquefied became easy after repeated trials, and was reasonably accurate. The concentration which caused liquefaction of practically all of the eggs in less than one minute was found, and then dilutions of the solution were made until the concentration was found which just caused a liquefaction of an estimated ninety-five per cent in one minute. A small per cent of the eggs was always more resistant than the rest, and for this reason it was more accurate to have ninety-five per cent as the constant to be liquefied in each case. Repetitions of the tests were very frequently made in order to verify results.

The following extracts from the notes on the experiments performed will illustrate in a manner how the results were obtained;

*Experiment 1.* — Urethane. A 1 mol. solution liquefied 99 per cent of the eggs in 30 seconds; a  $\frac{1}{2}$  mol., 5 per cent in 1 minute; a  $\frac{3}{4}$  mol., 95 per cent in 1 minute.

*Experiment 2.* — Chloretone. A  $\frac{1}{100}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{1}{200}$  mol., 5 per cent in 1 minute; a  $\frac{3}{400}$  mol., 95 per cent in 1 minute.

*Experiment 3.* — Butyl chloral hydrate. A  $\frac{1}{25}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{3}{100}$  mol., 95 per cent in 30 seconds; a  $\frac{1}{50}$  mol., 95 per cent in 1 minute.

*Experiment 4.* — Paraldehyde. A  $\frac{1}{25}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{1}{50}$  mol., 50 per cent in 1 minute; a  $\frac{3}{100}$  mol., 95 per cent in 1 minute.

*Experiment 5.* — Chloroform. A  $\frac{1}{25}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{1}{50}$  mol., 95 per cent in 1 minute.

*Experiment 6.* — Amyl alcohol. A  $\frac{1}{10}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{1}{20}$  mol., 50 per cent in 1 minute; a  $\frac{3}{40}$  mol., 85 per cent in 1 minute; a  $\frac{1}{50}$  mol., 95 per cent in 1 minute.

*Experiment 7.* — Acetone. A 2 mol. solution liquefied 99 per cent in 40 seconds; a 1 mol., 80 per cent in 1 minute; a  $\frac{3}{2}$  mol., 85 per cent in 1 minute; a  $\frac{1}{50}$  mol., 95 per cent in 1 minute.

*Experiment 8.* — Methyl alcohol. A 5 mol. solution liquefied 99 per cent in 40 seconds; a  $\frac{5}{2}$  mol., 30 per cent in 1 minute; a 4 mol., 98 per cent in 1 minute; a  $\frac{1}{4}$  mol., 95 per cent in 1 minute.

*Experiment 9.* — Isobutyric alcohol. A  $\frac{1}{2}$  mol. solution liquefied 100 per cent in 25 seconds; a  $\frac{3}{8}$  mol., 99 per cent in 40 seconds; a  $\frac{1}{4}$  mol., 95 per cent in 1 minute.



*Experiment 10.* — Ethyl alcohol. A 5 mol. solution liquefied 100 per cent in 25 seconds; a 3 mol., 99 per cent in 35 seconds; a 2 mol., 95 per cent in 1 minute.

*Experiment 11.* — Ether. A  $\frac{1}{2}$  mol. solution liquefied 99 per cent in 30 seconds; a  $\frac{1}{4}$  mol., 60 per cent in 1 minute; a  $\frac{3}{10}$  mol., 95 per cent in 1 minute.

*Experiment 12.* — Chloralamid. A  $\frac{1}{5}$  mol. solution liquefied 99 per cent in 1 minute; a  $\frac{3}{20}$  mol., 60 per cent in 1 minute; a  $\frac{6}{35}$  mol., 95 per cent in 1 minute.

*Experiment 13.* — Bromoform. A  $\frac{1}{125}$  mol. solution liquefied 100 per cent in 30 seconds; a  $\frac{3}{25}$  mol., 40 per cent in 1 minute; a  $\frac{1}{175}$  mol., 98 per cent in 1 minute; a  $\frac{1}{1250}$  mol., 95 per cent in 1 minute.

*Experiment 14.* — Chloral hydrate. A  $\frac{1}{4}$  mol. solution liquefied 100 per cent in 1 minute; a  $\frac{1}{20}$  mol., 10 per cent in 1 minute; a  $\frac{3}{40}$  mol., 85 per cent in 1 minute; a  $\frac{1}{40}$  mol., 95 per cent in 1 minute.

*Experiment 15.* — Hedonal. A  $\frac{1}{25}$  mol. solution liquefied 100 per cent in 40 seconds; a  $\frac{1}{50}$  mol., 50 per cent in 1 minute; a  $\frac{3}{100}$  mol., 99 per cent in 50 seconds; a  $\frac{1}{25}$  mol., 95 per cent in 1 minute.

*Experiment 16.* — Ethyl bromide. A  $\frac{1}{10}$  mol. solution liquefied 100 per cent in 1 minute; a  $\frac{3}{25}$  mol., 50 per cent in 1 minute; a  $\frac{1}{100}$  mol., 95 per cent in 1 minute.

In Table II are presented the solutions and their concentrations which liquefied an estimated ninety-five per cent of the eggs in one minute. The solutions are arranged in the order of their liquefying power, the most active being placed first.

A comparative study was made of the toxicity for *Fundulus heteroclitus*, of the solutions used in the work on the liquefaction of the eggs. The method of work was very simple. The concentration of a solution was found, which was fatal to a fish in about five or ten minutes. Then one hundred c.c. of this concentration of the solution and the same amount of five or six other concentrations of the same solution, each more dilute than the one just before it, were placed in finger bowls. Into each bowl were then placed three small fish, usually about of one size; but this was of little or no importance. It was essential though that the fish be uninjured, as those which had the scales removed took up the narcotics more rapidly and succumbed earlier than the sound ones. Garrey<sup>1</sup> showed that *Fundulus* with the scales removed from the body died much sooner than the uninjured fish when both were put in distilled water. The time of the putting

<sup>1</sup> GARREY: Biological bulletin, 1904, viii. p. 227.

of the fish into the solution and the time at which the gill arches ceased to move were carefully observed and recorded. It was found that the temperature of the solutions was an important factor; consequently the solutions which were kept at room temperature were

TABLE II.

No.	Compound.	Concentration.		
		gm.-mol $\frac{7}{1250}$	gm.-mol	per cent.
1	Bromoform . . . . .	$\frac{7}{1250}$	0.0056	0.1005
2	Chloretone . . . . .	$\frac{3}{400}$	0.0075	0.2641
3	Butyl chloral hydrate . .	$\frac{1}{50}$	0.02	0.0384
4	Chloroform . . . . .	$\frac{1}{50}$	0.02	0.2369
5	Hedonal . . . . .	$\frac{2}{75}$	0.027	0.3470
6	Paraldehyde . . . . .	$\frac{3}{100}$	0.03	0.3933
7	Amyl alcohol . . . . .	$\frac{2}{25}$	0.08	0.6994
8	Chloral hydrate . . . . .	$\frac{7}{80}$	0.0875	1.4360
9	Ethyl bromide . . . . .	$\frac{10}{109}$	0.0917	1.0000
10	Chloralamid . . . . .	$\frac{6}{35}$	0.17	0.3274
11	Isobutyric alcohol . . . .	$\frac{1}{4}$	0.25	1.8355
12	Ether . . . . .	$\frac{3}{10}$	0.3	2.2056
13	Urethane . . . . .	$\frac{3}{4}$	0.75	7.1250
14	Acetone . . . . .	$\frac{8}{5}$	1.6	9.2176
15	Ethyl alcohol . . . . .	$\frac{2}{1}$	2.0	9.1400
16	Methyl alcohol . . . . .	$\frac{15}{4}$	3.75	11.9212

diluted with sea water at room temperature, and the experiments were carried out at the same temperature. The vessels containing the solutions and fish were kept covered with glass plates. Experiments were usually not prolonged over a few hours, as the more prolonged the experiment the greater the possible source of error. In the course of twenty-four hours, the temperature variation is considerable; the evaporation of some of the volatile solutions becomes appreciable; the accumulation of waste from the fish, although small, is a factor; the supply of oxygen is less adequate after a short time; and the individual variation in susceptibility is more pronounced, the longer the solution requires to effect anesthesia. The three fish in the

strongest concentrations used usually stopped breathing at practically the same time, while in the greater dilutions there were more individual variations in the susceptibility, and this increased the more the dilution increased.

In Table III is given a typical experiment which was taken from the protocols. This indicates very fairly the variations in the results. In those compounds which were less active than hedonal, there were wider variations in the concentrations used.

TABLE III.

Solution and concentration.	Temperature.	Time of putting fish in sol.	Time they stopped respiring.			Remarks.
			min. sec.	min. sec.	min. sec.	
10 c.c. $\frac{1}{25}$ mol. Hedonal + 90 c.c. sea water	21° C.	9.57 A. M.	10_00	10 00	10 00	Those in solutions still weaker were alive at 4 P. M.
9 c.c. $\frac{1}{25}$ mol. Hedonal + 91 c.c. sea water	..	..	10 11	10 19	10 19	
8 c.c. $\frac{1}{25}$ mol. Hedonal + 92 c.c. sea water	..	..	10 12	10 32	10 44	
7 c.c. $\frac{1}{25}$ mol. Hedonal + 93 c.c. sea water	..	..	10 23	10 55	11 00	
6 c.c. $\frac{1}{25}$ mol. Hedonal + 94 c.c. sea water	..	..	10 46	11 36		

In Table IV the results are given of the concentrations of each solution which produced anesthesia in fifteen to thirty minutes.

It will be seen from Table IV that even at the concentration used the individual variations in susceptibility to the drugs is a factor; the variation is not so great, however, but that the toxic doses can be compared with considerable accuracy. The temperature variation of the different experiments is slight, and is not sufficient to cause any great error in the results.

Table IV shows the effects of temperature on the narcotic action. Chloroform, urethane, and hedonal were used. The concentrations, the temperature, and the length of times the fish respired after being put into the solution, are shown.

From Table V it is seen that the higher the temperature the quicker the compounds produce narcosis. The chemical processes of the animals which live in sea water have their optimum action at a temperature but little above that of the sea water. Carlson<sup>1</sup> found that the heart muscle of *Limulus* contracted best at a temperature

<sup>1</sup> CARLSON: Personal communication.

not above the ordinary room temperature. The author found that the enzymes of the ova and sperm of starfish had their optimum action at or a little above the temperature of the sea water in summer time.

TABLE IV.

No.	Compound.	Molecular concentration.		No. of minutes fish respired in sol.			Temperature.	Fish put in fresh sea water as soon as gill arches stop.
		gm.-mol 1250	gm.-mol 1000					
1	Bromoform . . . .	$\frac{1}{1250}$	0.0008	12	22	26	20	Very few revive.
2	Chloretone . . . .	$\frac{1}{1000}$	0.001	18	20	25	19½	Revive readily.
3	Hedonal . . . . .	$\frac{1}{250}$	0.004	14	22	25	19¾	“ “
4	Paraldehyde . . . .	$\frac{3}{500}$	0.006	15	20	21	19¾	“ “
5	Chloroform . . . .	$\frac{1}{125}$	0.008	17	22	23	19¾	A revival rare.
6	Butyl chloral hydrate	$\frac{7}{500}$	0.014	12	24	24	22	Very slow to revive.
7	Amyl alcohol . . . .	$\frac{1}{40}$	0.025	13	18	18	19½	Revive readily.
8	Iso butyric alcohol .	$\frac{3}{50}$	0.06	15	16	18	19¾	“ “
9	Ethyl bromide . . . .	....	....	..	..	..	....	
10	Chloral amid . . . .	$\frac{1}{10}$	0.1	25	40	40	19½	
11	Chloral hydrate . . .	$\frac{3}{20}$	0.15	15	17	21	20½	
12	Ether . . . . .	....	....	..	..	..	....	Omitted by mistake.
13	Urethane . . . . .	$\frac{1}{5}$	0.2	20	23	25	19½	Revive readily.
14	Acetone . . . . .	$\frac{3}{10}$	0.3	14	20	20	19½	“ “
15	Ethyl alcohol . . . .	1¼	1.25	12	25	25	20½	Very few revive.
16	Methyl alcohol . . . .	1½	1.5	18	18	18	19½	About one-half revive.

An increased temperature then causing more rapid narcosis probably does so by some change produced in the narcotic compound. Mathews<sup>1</sup> thinks that this can be explained on the basis of Nef's<sup>2</sup> bivalent carbon compound hypothesis for the reactions in organic chemistry. The reaction of these carbon compounds is by virtue of the two free bonds of the carbon. When the two bonds are united to each other, the compound is not active chemically. The activity of the compound is in proportion to the number of free bonds. Certain conditions increase the number of free bonds in solution. And

<sup>1</sup> MATHEWS: Ref. cited.

<sup>2</sup> NEF: Ref. cited.

one of the most important of these conditions is an increased temperature. A fish is anesthetized quicker, then, at a high temperature than at a low, by urethane, because the free bivalent carbon bonds are increased in number in the former condition; the increased

TABLE V.

Compounds.	Molecular concentration.		No. of minutes respire in solution.			Temperature.
	gram 100	gram 0.01				
Chloroform	100	0.01	13	14	14	22½
“	100	0.01	17	17	23	19¾
Urethane	25	0.08	2	2	2	36
“	“	“	16	16	16	32
“	“	“	17	17	17	31
“	“	“	30	30	30	28
“	“	“	40	44	51	24¾
“	“	“	60	65	73	19½
Hedonal	250	0.04	3	3	3	21½
“	“	“	22	22	25	19¾

number of bonds causes the compound to unite more readily with the constituents of the cell, and thus interfere with the processes of the cell proportionately sooner.

By a comparative study of Tables II and IV there will be found a very good parallelism in the order of the drugs, which are arranged according to their action in the two cases. There are no wide divergences. Bromoform, chloretone, amyl alcohol, ethyl bromide, chloral amid, ether, urethane, acetone, ethyl alcohol, and methyl alcohol occupy the same relative position in each table. Ethyl bromide and ether were not used on the fish. There was not sufficient solution of the former, and the latter was omitted by an oversight. Differences are noted as follows: chloroform in Table II is No. 4, and in Table IV it is No. 5; butyl chloral hydrate, which is No. 3 in Table II, is No. 6 in Table IV; hedonal, which is No. 5 in Table II, is No. 3 in Table IV; paraldehyde, which is No. 6 in Table II, is No. 4 in Table IV; chloral hydrate, which is No. 8 in Table II, is No. 11 in Table IV; isobutyric alcohol, which is No. 11 in Table II, is No. 8 in

Table IV. In no case do the positions of a compound in the two tables vary more than three places; and those which vary, have on either side solutions of about equal concentration. These minor variations may well be due to experimental errors, as in neither the liquefaction nor the anesthetizing experiments was the end point especially delicate.

#### SUMMARY.

The anesthetics and narcotics at certain concentrations cause profound changes in the eggs of starfish. This change appears to be a partial liquefaction. The power of the compounds in bringing this about is indicative of their power as narcotics; *i. e.*, the narcotic substance which produces liquefaction of the eggs in a dilute solution will also, in small amounts, produce narcosis.

The relative narcotic or anesthetizing power of sixteen compounds is shown in Tables II and IV; the differences between the tables are attributed to unavoidable experimental errors.

The anesthetics and narcotics do not cause the liquefaction if they are sufficiently concentrated or sufficiently diluted. The concentrated solutions cause a change which has the appearance of a coagulation.

The most important rôle of the lipoids in bringing about anesthesia probably is one of accumulation. If they are concerned with the essential processes of the cell, then their part is most likely a broader one.

Anesthesia is very possibly the result of an inhibition by the compounds, of the enzymotic processes of the cell, as suggested by Neilson and Terry.

Mathews's idea that the anesthetics produce their results by their influence upon the respiratory elements — the bivalent carbon compound — of the cell is a tenable one.

Nef's bivalent carbon hypothesis may help to explain the more rapid narcosis when the temperature is slightly raised.

It is a pleasure for me to acknowledge the helpful suggestions of Professors Lyon, Mathews, and Neilson.





# ON THE MECHANISM OF CO-ORDINATION AND CONDUCTION IN THE HEART WITH SPECIAL REFERENCE TO THE HEART OF LIMULUS.

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## I. THE RATE OF CONDUCTION IN THE INTRINSIC NERVES OF THE HEART.

THE statement is frequently made in text-books, as well as in special articles treating of the physiology of the heart, that the rate of conduction of the contraction wave between the auricles and the ventricles as well as in the auricles and the ventricles themselves is too slow to be a conduction in nerve fibres or a nerve plexus. Engelmann<sup>1</sup> estimates the rate of conduction in the frog's auricle to be 15 to 20 cm. per second. In the frog's ventricle the conduction appears to be even less rapid. In the ventricle of the California hagfish (*Bdellostoma*) I found the rate of conduction to be as low as 25 mm. per second.<sup>2</sup> The experiments of Schlüter<sup>3</sup> on the changes in the electrical tension of the mammalian ventricle in activity also indicate a rate of conduction very much slower than in the motor nerves to the skeletal muscles, or only 2 to 4 m. per second. Bethe's<sup>4</sup> measurements of the rate of conduction in the mammalian auricle, making use of the direct graphic method, indicated a rate even lower or from  $1\frac{1}{4}$  to  $2\frac{1}{4}$  m.

Even on the theory that the co-ordination of the heart takes place in the muscular tissue, the delay of the contraction at the auriculo-ventricular junction might be interpreted as due, at least in part, to the longer latent period of the ventricular muscle, but the usual

<sup>1</sup> ENGELMANN: *Archiv für die gesammte Physiologie*, 1894, lvi, p. 160.

<sup>2</sup> CARLSON: *Zeitschrift für allgemeine Physiologie*, 1904, iv, p. 259.

<sup>3</sup> SCHLÜTER: *Archiv für die gesammte Physiologie*, 1902, lxxxix, p. 87.

<sup>4</sup> BETHE: *Anatomie und Physiologie des Nervensystems*, 1903, p. 439.

interpretation is that this pause is caused by the slower conduction in the muscular tissue connecting these two parts of the heart. On this assumption I estimated the rate of the conduction in the canalis auricularis of the hagfish to be as low as 3 to 4 mm. per second. Even with due allowance for the fact that the measurements of the conduction rate in the heart have been made on the heart under more or less artificial conditions, so that the conduction was in all probability slower than the normal, there can be no doubt that the conduction in the vertebrate heart is considerably less rapid than in the motor nerves to the skeletal muscles of the same animal, probably even slower than the conduction of the contraction in the skeletal muscles themselves. But the argument from this fact in favor of the myogenic theory of conduction rests upon the erroneous assumption that all nervous paths in the same animal conduct with the same rapidity. Engelmann recognizes, to be sure, that the rate of conduction in nerves varies in different animals, citing Fredericq's measurements of the conduction in the motor nerves of the crayfish as an example of slow conducting nerves (6 m. per sec.). Bethe has recently subjected that assumption to rigid criticism, pointing out that the conduction in the nerves of some of the invertebrates is much slower than that in the mammalian heart. But all data so far at hand have only an indirect bearing on this question. No one has yet measured the rate of conduction in the intrinsic heart nerves under conditions excluding the possibility of muscular conduction. It is well known, however, that in the more complex paths in the central nervous system the impulse travels slower than in the peripheral motor nerves of the same animal. I have shown that the rapidity of conduction of the impulse stands in a direct relation to the rapidity of contraction of the muscle supplied by the respective nerves.<sup>1</sup> In other words, even the peripheral motor nerves of the same animal exhibit constant differences in the rate of conduction according to the character of the muscle which they supply. Since reporting those results I have obtained further data in the support of this conclusion. These data will soon be published. If this relation between the rate of conduction in the nerve and the rate of contraction in the muscle shall prove to hold good for all neuro-muscular mechanisms, it follows that the conduction in the motor nerves and the nervous plexus of the visceral organs is approximately as much slower than that of the nerves to the skeletal muscles as the visceral and the skeletal muscles differ in

<sup>1</sup> CARLSON: This journal, 1904, x, p. 401.

rapidity of contraction. So far as the intrinsic nervous tissue of the heart and the viscera of the vertebrates are concerned, this is only a working hypothesis, as we have, so far as I know, no data touching the rate of conduction in these structures. In the *Limulus* I have succeeded in obtaining accurate measurements of the conducting rate in the intrinsic heart nerves, so that it may be compared with that in the peripheral motor nerves, and it may be stated at the outset that the above stated hypothesis proved to be true. *The rate of conduction in the intrinsic motor nerve plexus of the heart is from eight to ten times less than that in the peripheral motor nerves.*

The intrinsic cardiac nerves of the *Limulus* heart and their relations to the heart muscle have been described in detail by Patten and Redenbough, and described and figured briefly in my papers on automaticity and the phenomenon of inhibition in the *Limulus* heart.<sup>1</sup> A further description of them is therefore superfluous in this connection. It will suffice to recall that the ganglion or automatic centre of the heart rhythm is connected with the muscle by a motor nerve plexus on the dorsal side of the heart. This plexus is collected into two main nerves, one in each lateral angle of the heart. Both the ganglion and the nerve plexus lie on the surface of the heart muscle. Now, inasmuch as many motor fibres pass from the ganglion into the middle and posterior region of the heart, by means of the lateral nerves, to the muscle of the anterior end of the heart, thus affording a motor nervous path of several centimetres' length that can be isolated for experimental purposes, it occurred to me that by making use of the anterior end of the heart and the lateral nerves a workable nerve-muscle preparation might be obtained in which the rate of conduction in the nerve could be measured by the ordinary graphic method of Helmholtz. This proved to be entirely correct. Fig. 1, *I* represents diagrammatically the preparation of the first heart segment and the lateral nerve on one side for such experiments. The heart of the largest specimens were used. The lateral nerve was isolated from the ganglion and the muscle for the distance between the fifth up to the middle of the second segment. The heart muscle, the median nerve cord, and the lateral nerve on the opposite side were severed in the middle of the second segment, leaving the preparation as shown in Fig. 1, *I*. The anterior segment was supported and connected with the recording lever either by means of platinum hooks passed into opposite sides, as shown in the diagram, or by means of ligatures

<sup>1</sup> CARLSON: This journal, 1904, xii, p. 67; 1905, xii, p. 471; xiii, p. 217.

secured to the first pair of lateral arteries. The latter method was the one usually employed. In some of the experiments both of the lateral nerves were thus isolated and placed on the electrodes, as the stimulation of both nerves usually gave a stronger contraction than that produced by the stimulation of either nerve alone. There are individual variations in the size of the lateral nerves of the two sides,

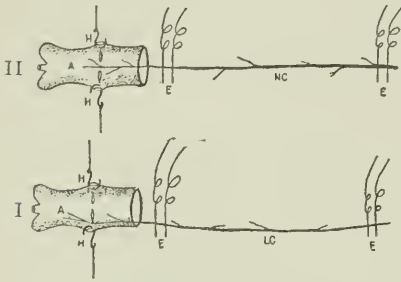


FIGURE 1. — Diagrams showing preparation of heart and intrinsic heart nerves of *Limulus* for measuring the rate of conduction of the impulse in the nerves. *I*, preparation of the lateral nerve. *II*, preparation of the median nerve cord. *A*, anterior two segments of the heart. *E*, electrodes. *H*, hooks for suspending the heart and connecting it with the recording lever. *LC*, lateral nerve. *NC*, median nerve cord.

as well as in the number of fibres passing in the lateral nerves as compared to that in the median nerve cord. For the experiments on the lateral nerves those specimens were selected in which the lateral nerves were relatively large. It is not necessary to remove the ganglion or median nerve cord from the preparation shown in Fig. 1, *I*, that is, from the first two segments, in order to produce quiescence in this part. I have pointed out in a previous paper that the ganglion at this, the aortic, end of the heart contains only a few ganglion cells and exhibits little or no automaticity when isolated from the portion of the nerve cord behind. Isolating the lateral nerves from the nerve cord and transecting the latter in the second segment thus gives a preparation perfectly quiescent, except on direct stimulation or on stimulation of the lateral nerves. The anterior heart segment from a large specimen is sufficiently strong to lift a relatively heavy lever, so the preparation offers no difficulty in that line.

I was interested in comparing the rate of conduction in the lateral nerves with that in the nerve cord. For this purpose the heart was prepared as shown in Fig. 1, *II*, the nerve cord alone being left in connection with the first two segments. This muscle-nerve-cord preparation usually exhibited the disadvantage of continuing in rhythm. This was invariably the case if the nerve cord included that of the fifth and sixth segments. If the cord was transected between the fourth and the fifth segment, a quiescent preparation was sometimes obtained. Some measurements were also made on active preparation by stimulating the nerve cord distally and proximally at corresponding points on the diastolic curve.

The nerve-muscle preparation has this drawback, that there are a less number of motor fibres at the point of application of the distal than at the point of application of the proximal electrodes. This is particularly true of the preparation involving the lateral nerves, because some of the fibres that pass from the nerve cord to the muscle anteriorly run in the cord for some distance before passing into the plexus and the lateral nerves. Because of this fact the recording segment tends to contract more strongly when the lateral nerves are stimulated at a point nearer than at a point farther away from the muscle. Precautions were, of course, taken that no escape of the electrical current directly to the muscle might take place on the near stimulation. This might be a source of error in calculating the rate from the difference between the latent periods, because the stronger contraction would probably lift the lever from the base line more rapidly. Thus the calculated rate would be less than the actual. In some preparations this difference in the response to distal and proximal stimulations was insignificant or not in evidence, and in the preparation in which it was marked or constant, I endeavored to overcome it by using slightly weaker stimuli for the near stimulation. It goes without saying that in the course of these experiments many pairs of records were obtained, showing considerable difference in the magnitude of the contractions. But by excluding all records in which this discrepancy amounted to more than 0.1 to 0.2 mm. (one to three mm. as magnified by the lever) I think that this source of error does not influence the results.

The heart of *Limulus* will respond to the stimulation of the lateral nerves with single induced shocks. But these have to be so very strong in order to be effective that it is not possible to determine the distance of nerve between any two points of actual stimulation of the nerve. Hence, single induction shocks were abandoned, and a series of relatively weak interrupted currents of from  $\frac{1}{10}$  to  $\frac{1}{5}$  second duration was used as stimulus. This gave good results, although even to this form of stimulus the intrinsic heart nerves appear to be less irritable than are the motor nerves to the limbs and much less than the sciatic nerve of the frog.

It goes without saying that the greatest care was taken in isolating the lateral nerves or the nerve cord so as not to injure them and thus diminish (or possibly increase) the rate of conduction. The nerve cord is more readily isolated than the lateral nerves, but with sufficient care even the latter may be isolated without any, or, at the worst,



with but slight injury. As a check on this point some measurements were taken with the lateral nerve isolated from the muscle only at the point of application of the electrodes. These measurements gave values corresponding to those obtained with the nerves completely isolated.

TABLE I.

Rate of conduction of the impulse in the intrinsic heart nerves (lateral nerves) of *Limulus*.  
Detail record of Experiment No. 4, Table III.

Latent period in fifths of a second.		Latent period in fifths of a second.	
Distal.	Proximal.	Distal.	Proximal.
1.5	0.9	1.7	1.0
1.7	0.9	1.5	1.1
1.7	1.0	1.7	1.0
1.7	1.1	1.6	1.0
1.5	1.0	1.7	1.1
1.4	1.1	1.5	1.1
Average . . . . .		1.64	1.02
Difference in latent time, 0.13 second. Length of nerve, $4\frac{1}{2}$ cm. Rate of conduction, 36 cm. per second.			

The heart and the heart nerves will live for several days after removal from the body if protected from evaporation and kept at a temperature of  $10^{\circ}$ – $12^{\circ}$  C. But these experiments were all made on fresh preparations, the room temperature varying from  $18^{\circ}$  to  $22^{\circ}$  C.

Two series of experiments on the lateral nerves (Tables I and II) and one on the nerve cord (Table IV) are given in detail, the remainder only in summary (Tables III and V). The measurements on the cord and the lateral nerves are tabulated separately for the purpose of comparison. It will be seen from Tables I, II, and IV that the variation in the latent period on any two consecutive stimulations of the nerve at the same point is considerable, but it is in reality small in view of the large time element involved in the whole.

The extremes of the thirteen experiments on the lateral nerves (Table III) are 21 and 55 cm. per second respectively, with an average of 40 cm. per second. The greater number of the experiments fall between 40 and 50 cm. per second. It seems safe to conclude that these fig-

TABLE II.

Rate of conduction of the impulse in the intrinsic heart nerves (lateral nerves) of *Limulus*.  
Detail of Experiment No. 5, Table III.

Latent period in fifths of a second.	
Distal.	Proximal.
2.2	1.6
2.4	1.5
2.7	" 1.7
2.8	1.5
2.6	1.5
—	1.5
—	1.5
Average 2.50	1.55
Difference in latent time, 0.19 second. Length of nerve, 5.5 cm. Rate of conduction, 28.7 cm. per second.	

ures represent the maximum rapidity of conduction in the intrinsic motor nerves in the *Limulus* heart. Whatever other complicated mechanisms and conducting paths the nerve cord may contain, an inspection of Tables III and V shows that the nerve cord also contains motor fibres to the muscle that conduct with the same rate as those in the peripheral plexus and lateral nerves.

We concluded that 40 to 50 cm. per second is the maximum rate of conduction. It may, however, be apparently much less even in hearts that continue in rhythmic activity. This point will be referred to again when the conduction in the heart nerves is compared with that in the peripheral motor nerves. But it may be stated in this connection that the conduction in the heart nerves may be much

slower than 40 cm. per second under experimental conditions or in preparations taken from specimens in poor condition. I have excluded five series of measurements from the summary in Table III, because I

TABLE III.

Rate of conduction in the intrinsic heart nerves (lateral nerves) of *Limulus*.  
Summary of the measurements.

No. of experiment.	No. of pairs of records.	Transmission time in seconds.	Length of nerve in cm.	Rate in cm. per second.
1	4	0.14	3.0	21.0
2	3	0.10	4.0	40.0
3	5	0.11	4.5	40.0
4	12	0.13	4.5	36.0
5	5	0.19	5.5	28.7
6	7	0.09	4.0	44.0
7	6	0.12	5.0	41.5
8	3	0.08	4.0	48.0
9	7	0.11	5.5	55.0
10	3	0.085	4.0	48.0
11	3	0.08	3.5	43.7
12	4	0.085	4.0	48.0
13	7	0.08	3.0	37.5
Average rate, 40.9 cm. per second.				

knew the preparations to be in poor condition or injured in some way. These five preparations showed a rate of conduction of from 5 to 15 cm. per second. A pair of records from one of these series is reproduced in Fig. 3. It will be seen that the muscle in this case contracts much slower than under normal conditions (Fig. 2).

Experiments Nos. 1 and 5 are included in Table III because I had no evidence that these preparations were not in good condition, save the relatively slow rate of conduction.

TABLE IV.

Measurements of the rate of conduction in the intrinsic nervous plexus (median nerve cord) in the heart of *Limulus*. Detail of Experiment No. 1, Table V.

Latent period in fifths of a second.	
Distal.	Proximal.
1.3	0.6
1.3	0.7
1.2	0.6
1.2	0.6
1.3	0.6
1.2	0.6
1.0	0.5
Average 1.2	0.6
Difference in latent periods, 0.13 second. Length of nerve cord, 4.5 cm. Rate of conduction, 32 cm. per second.	

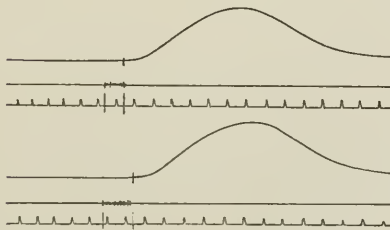


FIGURE 2.—Tracings from the contraction of the first heart segment on stimulating the lateral nerve at two levels. Distance between the distal and the proximal electrodes, 5.5 cm. Difference in latent periods, 0.13 second. Rate of conduction, 38.5 cm. per second. Time marker records  $\frac{1}{3}$  second.

## II. THE RATE OF CONDUCTION IN THE PERIPHERAL MOTOR NERVES (THE AMBULACRAL NERVES) OF LIMULUS.

The measurement of the rate of conduction in the motor fibres of the nerves to the ambulacral appendages proved to be a relatively easy task. The fourth pair of ambulacral appendages are the longest.

TABLE V.

Summary of measurement of the rate of conduction in the intrinsic nerve plexus (median nerve cord) of the heart of *Limulus*.

No. of experiment.	No. of pairs of records.	Transmission time in seconds.	Length of nerve cord in cm.	Rate in cm. per second.
1	7	0.13	4.5	32
2	7	0.10	4.0	40
3	2	0.18	5.5	33
4	6	0.10	5.0	50
5	4	0.10	4.0	40
6	2	0.10	4.5	45
7	8	0.12	5.0	42
8	3	0.10	4.0	40
9	8	0.085	4.0	48
10	5	0.10	5.0	50
11	6	0.075	3.0	40
12	7	0.08	3.0	37
Average rate, 41 cm. per second.				

Using one member of this pair of appendages from the largest specimens, one may obtain a length of nerve of from 10 to 14 cm. between the brain and the beginning of the last joint in the limb. The adductor muscle of the forceps or chela was used for recording, the appendage being prepared and held in position for the experiment in the manner shown in the diagram in Fig. 4. The nerve was isolated, together with the blood vessel or sinus surrounding it, from the brain clear down to the forceps. The branch to the abductor muscle of the forceps was usually severed so as to exclude any error in the

records that may be caused by the antagonistic action of the muscle. But even when the nerve to the abductor is left intact and is stimulated simultaneously with the fibres to the adductor muscle, the

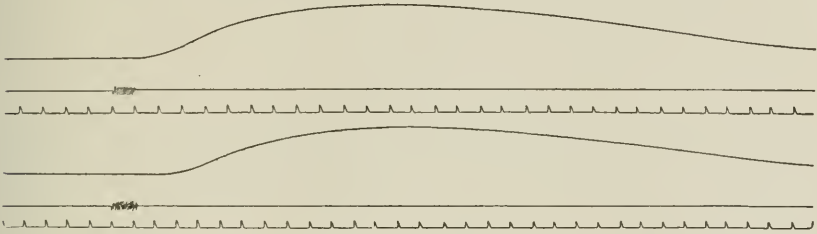


FIGURE 3.—Tracings of the contractions of the anterior end of the heart on stimulation of the lateral nerve at two levels. Distance between distal and proximal electrodes, 3 cm. Difference in latent periods, 0.20 second. Rate of conduction, 15 cm. per second. Time,  $\frac{1}{5}$  second. These records are from a preparation in poor condition.

latter muscle is so much stronger that the influence of its antagonist is hardly in evidence. The body of the forceps was held in position by a clamp in the manner shown in the diagram, a thread attached to the other prong passing to the recording lever.

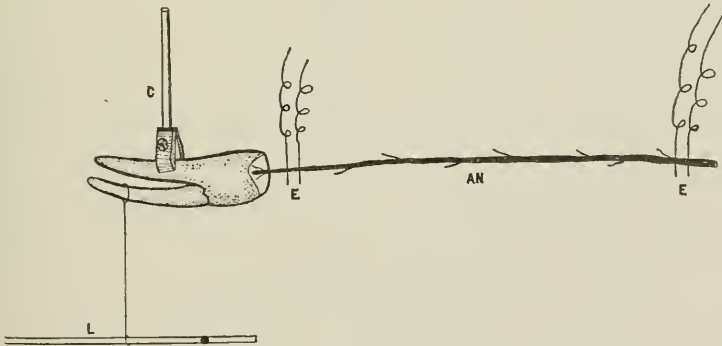


FIGURE 4.—Diagram illustrating the preparation and arrangement of one of the ambulacral appendages of *Limulus* for the purpose of measuring the rate of conduction in the motor nerve. *AN*, ambulacral nerve. *C*, clamp for holding the chela in position in the moist chamber. *E*, electrodes. *L*, recording lever.

The only difficulty offered by this preparation for these experiments is that the adductor and abductor muscles frequently pass into an almost rhythmic activity, closing and opening the forceps. This is probably caused in part by unavoidable injuries in the dissection, but the mere touching of either prong is sufficient to cause the



adductor muscle to contract even after the forceps have been severed from the rest of the appendage, and consequently also from the central nervous system. The mere weight of the lever sufficed to produce this effect in some of the preparations. But a sufficient number of quiescent preparations were obtained to secure enough

TABLE VI.

Rate of conduction of the nervous impulse in the ambulacral (limb) nerves of *Limulus*.  
Detail of experiment No. 12, Table VII.

Latent period in seconds.	
Distal.	Proximal.
0.065	0.036
0.060	0.037
0.065	0.035
0.060	0.035
0.060	0.034
0.065	0.038
0.065	0.037
0.062	0.036
0.066	0.038
Aver. 0.063	0.036
Difference in latent periods, 0.027 second.	
Length of nerve, 9.5 cm.	
Rate of conduction of the impulse, 351 cm. per sec.	

data on the conduction rate. Single induction shocks of relatively low intensity served as the stimulus.

Table VI contains the detail measurements of a typical experiment on the ambulacral nerve-muscle preparation. An inspection of this table shows that the variations in the latent period of two or more consecutive records is not great. The fairly close agreement in the values obtained in the fourteen experiments summarized in Table VII goes to show that the average of  $3\frac{1}{4}$  to  $3\frac{1}{2}$  m. per second comes very close to being the actual rate in these nerves. In other

words, the rate of conduction in the peripheral motor nerves to the limb muscles is from eight to ten times more rapid than that in the intrinsic motor nervous plexus in the heart. This incontrovertible fact in *Limulus*, it appears to me, weakens, not to say renders

TABLE VII.

Summary of measurements of the rate of conduction of the impulse in the ambulacral nerves of *Limulus*.

No. of experiment.	No. of pairs of records.	Transmission time in seconds.	Length of nerve in cm.	Rate in cm. per second.
1	3	0.020	7.5	375
2	4	0.025	8.0	320
3	7	0.034	10.0	295
4	5	0.022	8.5	383
5	3	0.030	9.0	297
6	3	0.030	10.0	333
7	2	0.030	10.0	333
8	3	0.040	12.0	300
9	2	0.025	8.5	340
10	4	0.040	13.0	325
11	3	0.040	13.0	325
12	9	0.027	9.5	351
13	7	0.035	11.5	325
14	2	0.024	7.0	292
Average rate, 328 cm. per second.				

untenable, Engelmann's argument in favor of the muscular conduction in the heart of vertebrates from the relatively slow rate of propagation of the contraction wave, based on the erroneous assumption that different nervous mechanisms in the same animal may not exhibit the difference in the rate of conduction that has been found to exist between the heart and the motor nerves to skeletal muscles. If the measurements by Schlüter and Bethe of the rate of conduction in the mammalian heart (2 to 4 m. per second) do not fall too wide of the mark, we have the same relation in the mammals as in *Limulus*; that

is, the conduction in the peripheral motor nerves is eight to ten times (30 to 40 m. per second) faster than the conduction in the heart. But this ratio does not hold good for the cold-blooded vertebrates (frog, hagfish), the conduction in the heart of these animals being relatively slower in comparison to that in the spinal nerves.

### III. THE MECHANISM OF CONDUCTION IN THE HEART WHEN IN A STATE OF WATER RIGOR.

While the neurogenic nature of conduction and co-ordination in the *Limulus* heart cannot be questioned, it is a fact that the results of the more recent investigations touching the same questions in the vertebrates are being interpreted as strengthening the myogenic theory. Let us see with what justification. The uniform presence of His's auriculo-ventricular strand of muscle fibres in many mammals is firmly established by the recent works of Retzer, Bräunig, Humblet, and Tawara.<sup>1</sup> Retzer is unable to affirm that muscular connections between the auricles and the ventricles do not also exist in the region of the auriculo-ventricular groove; but the main connecting bundle is in the septum. His's earlier conclusion that the region of this muscle bundle in the septum is the one concerned in the co-ordination between the auricles and the ventricles has also been recently confirmed by Humblet, Hering, and Erlanger.<sup>2</sup> Humblet and Hering produce permanent inco-ordination between the auricle and the ventricles by severing the region of the muscular bundle or "block fibres." Erlanger produces temporary inco-ordination of the same type by compression of this region of the septum. Erlanger's experiments are the more conclusive, as he is able by releasing the compression to restore the co-ordination, which, of course, cannot be done when the septum is transected. One of the first symptoms of a mammalian heart being under abnormal conditions is the loss of auriculo-ventricular co-ordination, and one might have objected to the conclusions of His, Humblet, and Hering that the inco-ordination might be due to other abnormal conditions attendant upon the opera-

<sup>1</sup> RETZER: *Archiv für Anatomie*, 1904, p. 1; BRÄUNIG: *Archiv für Physiologie*, 1904, p. 1; HUMBLET: *Archives internationales de physiologie*, 1904, i, p. 278; TAWARA: *Centralblatt für Physiologie*, 1905, xix, p. 298.

<sup>2</sup> HUMBLET: *Loc. cit.*; HERING: *Archiv für die gesammte Physiologie*, 1905, cvii, p. 97; cviii, p. 267; ERLANGER: *Centralblatt für Physiologie*, 1905, xix, pp. 9, 270.

tion rather than to the transection of the "block fibres." The results of Erlanger place it beyond doubt that in mammals (dog) the auriculo-ventricular co-ordination is effected by the region of His's muscle strand and by this region alone. Apart from this auriculo-ventricular muscular connection in the septum the mammalian auricles and the ventricles are connected by a superficial nervous plexus containing the augmentor nerves and possibly also by isolated muscle fibres in the auriculo-ventricular groove.

These experiments of producing auriculo-ventricular inco-ordination by sectioning or compressing the region of the auriculo-ventricular muscle bundle decide this one thing, that that region is the only one concerned in the co-ordination, but they do not decide whether the conduction takes place in the muscle fibres and the peculiar "Purkinje fibres" described by Tawara or in the nerve fibres of the intra-muscular nerve net. They would, indeed, be decisive in case no nerves or nerve plexus were present in the region severed or compressed. That was, to be sure, the contention of His. The presence of nerve fibres to and about the auriculo-ventricular muscle fibres or "block fibres" in cold-blooded vertebrates was rendered necessary by Gaskell's experiments showing that the power of conduction (assuming the myogenic theory) of these fibres is influenced by the extrinsic cardiac nerves. Granted that these muscle fibres are the ones concerned in the conduction, how could this conductivity be influenced by nerves unless these nerves passed among and to the muscle? In the frog's heart Tschermak<sup>1</sup> has recently demonstrated this intra-muscular nerve net by the method of Golgi. According to Tschermak, the auriculo-ventricular muscle strands are just as closely surrounded by this nervous network as the muscle fibres in the auricles and the ventricle. What is the condition in the mammals? According to the preliminary report of Dr. Tawara, the "bundle of His" is in the mammals investigated (calf, sheep) richly interwoven with nerve fibres, on the course of which ganglion cells are present.<sup>2</sup> Now, this being the case both for cold and warm blooded vertebrates, I fail to see how the transection or compression of this region of the septum comes any closer to deciding between the neurogenic and the myogenic theories than the transection or compression of any part of the auricles or the ventricles themselves. The solution of this question must evidently be sought by other methods.

<sup>1</sup> TSCHERMAK : *Centralblatt für Physiologie*, 1905, xix, p. 301.

<sup>2</sup> TAWARA : *Centralblatt für Physiologie*, 1905, xix, p. 298

The heart beat is frequently described as a "peristaltic wave of contraction" passing from the great veins to the aortic end of the heart, the base of the ventricle or ventricles contracting before the apex.<sup>1</sup> But even an advocate of the myogenic theory like Engelmann is forced to admit that the heart walls can conduct without contracting or being able to contract. On vagus stimulation the auricles may be brought to a complete standstill while the ventricle continues under the influence of the sinus, or the ventricle may respond to the stimulation of the quiescent auricle although the latter remains quiescent. Gaskell, and more recently Hering,<sup>2</sup> have objected to the conclusion that in these experiments the auricles were in reality quiescent, it being possible that contractions too feeble to be detected even by the aid of a powerful lens actually pass over the auricles. Contractions too feeble to be detected can of course neither be proved nor disproved, and are therefore outside the pale of physiological discussions. But there are other and even stronger reasons, it seems to me, forcing us to admit that the heart wall may conduct without contracting. In the heart of the cold-blooded vertebrates the base of the ventricle appears to contract before the apex, as demanded by the conception of the heart beat as a peristaltic wave. But this is not always the case in the mammalian heart. Fredericq<sup>3</sup> found that in the systole of the dog's ventricle the apex first became electro-negative to the base; that is, the contraction started at the apex. Waller and Reid<sup>4</sup> found that the apex (dog) may precede the base, or the base the apex, in electro-negativity during systole. In man Waller found a diphasic action current, the ventricular apex always first becoming negative to the base. These conclusions rest on observations by means of the galvanometer and the capillary electrometer as well as by the direct graphic registration of the contraction. Schlüter,<sup>5</sup> making use of two nerve-muscle preparations (frog) as indicators of the changes in the electrical tension in the heart of the dog, comes to

<sup>1</sup> ENGELMANN and GASKELL have construed this resemblance of the heart beat to the peristalsis of the digestive tract as an argument in favor of the myogenic theory, assuming that the intestinal peristalsis is myogenic. The recent researches of BAYLISS and STARLING, and MAGNUS have shown that the movements of the digestive tract are neurogenic in their nature.

<sup>2</sup> HERING: *Archiv für die gesammte Physiologie*, 1901, lxxxvi, p. 533.

<sup>3</sup> FREDERICQ: *Bulletin de l'académie royale de Belgique*, 1887, xiii.

<sup>4</sup> WALLER and REID: *Philosophical transactions*, 1888, clxxviii, p. 215; 1889, clxxx, p. 169.

<sup>5</sup> SCHLÜTER: *Archiv für die gesammte Physiologie*, 1902, lxxxix, p. 87.



the same conclusion as Waller and Reid: that the apex sometimes starts to beat before the base, at other times the base starts to beat before the apex, or the ventricle may beat simultaneously in all its parts. These three conditions may all be found in the same heart. Bayliss and Starling,<sup>1</sup> investigating the same question in the mammals, came to a slightly different conclusion, that in the injured heart the base of the ventricle always becomes negative to the apex at the beginning of the systole. In hearts under slightly abnormal conditions or injured in any way the electrical changes become reversed, the apex becoming negative to the base, as described by Fredericq, Waller and Reid, and Schlüter. Even if we admit the correctness of the conclusion of Bayliss and Starling that in the mammalian heart under perfectly normal conditions the negativity of the base is the initial electrical change in systole, the fact nevertheless remains that in hearts kept under conditions for the rhythm and the auriculo-ventricular co-ordination to continue for hours the ventricular apex may contract before the base; that is to say, under conditions when the ventricular contractions are still clearly caused by the changes accompanying the contraction of the auricles, the ventricular contraction begins, not in the part nearest the auricles, but in the parts farthest away. Additional evidence to the same effect has been produced by Engelmann in the action of the heart walls in the state of water rigor.<sup>2</sup> Using the frog's heart, Engelmann repeated Biedermann's experiments on the properties of skeletal muscle in condition of water rigor. The frog's auricles were bathed in hypotonic solutions until all power of contraction had been lost, the muscle being swollen and in a state of rigor; yet on stimulating the auricles in this condition the ventricle would respond, the auricles acting precisely as nervous tissue in conducting without contracting.

Hering,<sup>3</sup> on the contrary, found that the power of conduction and the power of contraction in the heart walls are not so independent as Engelmann's results would indicate. While Hering admits that the contractility decreases more rapidly than conductivity, he does not consider it proven that the heart tissue in sufficiently complete water rigor for all contractility to be lost may still conduct. Still, this would appear necessarily to be the case if it is true, as Hering admits, that contractility decreases more rapidly than conductivity;

<sup>1</sup> BAYLISS and STARLING: Proceedings of the Royal Society, 1892, 1, p. 211.

<sup>2</sup> ENGELMANN: Archiv für die gesammte Physiologie, 1894, lvi, p. 149.

<sup>3</sup> HERING: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 533.



for under these conditions the former would be abolished before the latter. This criticism might be made on Biedermann's and Engelmann's experiments and conclusions. It is by no means certain that in those of their preparations that showed conduction in the absence of contraction all the muscle cells in the interior of the heart wall or the sartorius muscle were in a state of rigor. In bathing a piece of muscle in water or hypotonic solutions the outer portion of the muscle becomes swollen and loses its contractility before the centre. Now, unless the bath is continued long enough for the centre to have

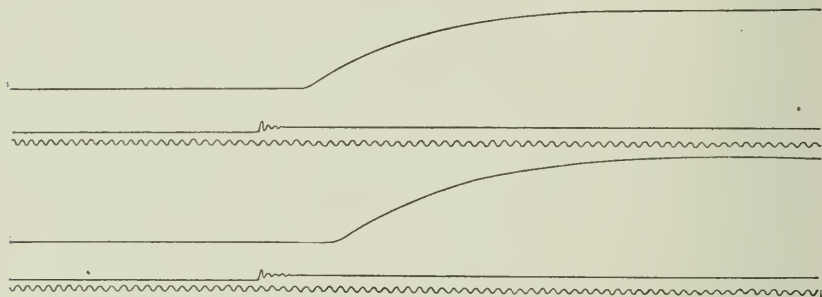


FIGURE 5. — Tracings from the contractions of the adductor of the forceps on stimulation of the ambulacral nerve near the brain and near the forceps respectively. Length of nerve between distal and proximal electrodes, 8 cm. Difference in latent time, 0.02 second. Rate of conduction, 400 cm. per second. Time, 100 d. v. per second.

reached the same state of rigor, one might readily obtain what might be interpreted as conduction in the absence of contraction, even if such a thing is actually impossible in the muscle; for the conduction would be carried out by the centre of the muscle, but the contraction of that portion might be unable to alter the form of the rigid exterior. So far as the experiment with muscle in water rigor is concerned, I am therefore inclined to agree with Hering that it is not proven that muscle conducts without contraction. But this admission does not apply to the experiments of Fredericq, Waller and Reid, Bayliss and Starling, Schlüter, Hofmann,<sup>1</sup> and Bethe. Unless we take recourse to the view that in these experiments "contractions too minute to be detected" pass over the auricular walls, there is no escape from the conclusion that the heart walls may conduct without contracting.

This fact may be interpreted in two ways. The conduction and the contraction in the heart may be concerned with two different

<sup>1</sup> HOFMANN: *Archiv für die gesammte Physiologie*, 1898, lxxii, p. 443.

tissues which are different in their resistance to the injurious action of water; or these two processes are concerned with the same tissue, but they are independent of one another to the extent that the one may be abolished or held in abeyance without impairing the other. If conduction in the heart takes place, not in the muscle but in the intra-muscular nerve plexus, it follows that in the water rigor of the heart it is this nervous network which retains its power of conduction while the muscle has entirely lost its contractility. This possibility has hardly been considered by Engelmann and Hering; nor has, so far as I know, anything been done to prove or disprove it.

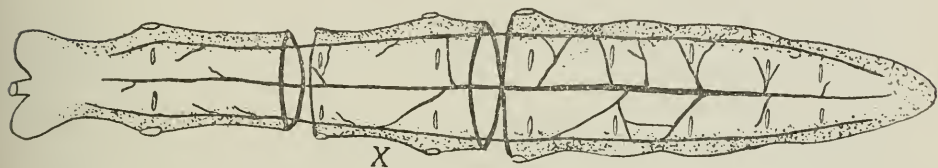


FIGURE 6. — Diagram (dorsal view) illustrating the preparation of the *Limulus* heart for studying the nature of conduction in water rigor. The heart muscle is transected and dissected away for a distance of  $\frac{1}{2}$  cm. in the region of the second and fourth heart segments, leaving the median nerve cord and the lateral nerves intact.

In the heart of *Limulus* this possibility can be put to a crucial test. For these experiments on the nature of conduction in water rigor the *Limulus* heart is prepared as shown by the diagram in Fig. 6. The lateral nerves and the median nerve cord are carefully isolated in the region of the second and the fourth segments, and in these two regions the heart muscle is completely transected and dissected away for a distance of half a centimetre. We have thus three muscular portions of the heart separated from one another and connected only by the lateral nerves and the median nerve cord. If the dissections are done carefully, so as not to injure these nervous connections, the three portions of the heart continue in perfect rhythm and synchrony for hours. It has been shown in previous papers that the median nerve cord is the automatic centre of the heart beat, and that the portion of this nerve cord exhibiting the greatest automatism is that of the fourth, fifth, and sixth segments of the heart. Thus, when the heart is transected in the region of the fourth segment, the anterior portion of the heart will exhibit little or no independent rhythm. In the heart prepared as just described the two anterior portions are therefore beating in response to impulses from a portion of the cord

in the posterior part of the preparation. The middle portion (Fig. 6, X), lateral nerves, nerve cord and all, can now be dipped into distilled water, or any solution desired, and the effects observed both on contractility and conductivity. If the nerves should cease to conduct at the same time or before the muscle has lost its contractility, the portion immersed in water and the portion anterior to it would cease to beat at the same time, but they would both retain their excitability to direct stimulation, though this might be diminished in the water-soaked segments. If, on the other hand, the injurious effect is greater or more rapid on the muscle than on the nerve, then the middle portion should lose its rhythm as well as its excitability to direct stimulation, while the anterior portion still continues to beat in synchrony with the rest of the heart, the impulses being conducted through the middle portions by the nervous network, although the muscle in this region is in complete water rigor. If this is the condition, it is evident that the nerves would also conduct the impulses to the segments in water rigor, although the latter would not be able to respond. The anterior portion, not being in water rigor, can thus be used for determining whether the nerves retain their conductivity or not. A series of experiments of this kind was carried out at the Woods Hole Laboratory last summer and repeated in our Chicago laboratory this fall, all of them yielding uniform results. These results may be briefly summarized as follows: *The distilled water injures the power of contraction in the muscle as well as the power of conduction in the nerves, but the nerves retain their conductivity for some time after the muscle has ceased its rhythm and lost its excitability to artificial stimulation.* These results are therefore identical with those obtained by Engelmann in the auricles of the frog, but in the *Limulus* heart the analysis of the mechanism involved can be carried further than Engelmann attempted to carry it in the frog's heart. That the muscle should show less resistance to the injurious effects of distilled water than do the nerves was entirely unlooked for at the beginning of the experiments. I expected to obtain the opposite results. The intrinsic nervous plexus in the *Limulus* heart is entirely similar to the nerve plexus in the heart of vertebrates. In both cases the network is composed of non-medullated nerve fibres, so that their resistance to the action of water cannot be ascribed to the protection of any myelin sheath.

The relative rapidity of loss of conduction in the nerve varies in different preparations. Hearts from animals in good condition ex-

hibited the greatest resistance. In these hearts the nerves would retain their conductivity, at least in part, for thirty to forty-five minutes in the continuous water-bath. The loss of conductivity is gradual. On removing this portion of the heart to plasma or sea water the conductivity is rapidly regained, sometimes within a minute or two, depending on how long the portion remained in the water after the conductivity was entirely lost. The muscle ceases to respond to the normal nervous impulse within fifteen or twenty minutes. Care must be taken in order to be sure that the muscle has entirely ceased to contract, because the action of the distilled water is greatest on the surface, coating the heart wall with a layer of muscle, whitish and swollen and in complete water rigor, while the interior of the heart wall is as yet little affected by the water, and the muscle there may continue in feeble rhythm, although no sign to that effect can be obtained by observing the exterior. At this stage of the rigor a strong interrupted current applied directly to this portion will produce a feeble, to all appearance, tonic contraction. In some preparations such a feeble tonic contraction can be obtained on direct stimulation with a very strong interrupted current even at the time the nerves have ceased to conduct the normal impulses, but in the majority of the preparations this was not the case. On replacing the water with plasma or sea water the nerves regain their conductivity, as has already been noted, but the recovery of the muscle is very slow and uncertain. The muscle may and does lose its water rigor, the whitish and swollen character disappears, the muscle resuming more of its normal hue and form, yet the rhythm very seldom returns; that is to say, the muscle fibres are for some reason not able to respond to the nervous impulses. Now, if we return this portion of the heart to the water-bath after the nerves have completely recovered their conductivity, the muscle, in case it has recovered its rhythm, loses its contractility and goes into rigor more rapidly than at the beginning of the experiment, while the nerves show almost as great resistance as at the beginning. In this way the nerves may conduct without almost any sign of injury from fifteen to twenty minutes with the muscle in complete rigor. By thus alternating between plasma and water several times the muscle is finally so greatly injured that it does not recover contractility at all, while the nerves conduct almost as perfectly as before the experiment began. The rate of conduction in the nerves when injured by the action of the water was not determined.

These experiments, it appears to me, are crucial. There can be no question that in the case of *the heart of Limulus in water rigor the heart walls will conduct without contracting. The conduction does not take place in the water-soaked muscle cells, but in the nervous network, which for some reason is less easily injured by the water than the muscle fibres themselves.* While these experiments are demonstrative only for the heart of *Limulus*, they also challenge the correctness of Engelmann's interpretation of conduction in the water rigor in the vertebrate heart. We have the same or similar muscular tissues in the *Limulus* heart and the heart of vertebrates. The intrinsic nervous tissue is similar in both, although we have as yet little direct knowledge of the function of this nervous system in the vertebrates. The behavior of the hearts in water rigor is also the same. These facts incline me to the view that the mechanism of conduction in water rigor in the vertebrate heart is the same as in the heart of *Limulus*.



# THE REVIVAL OF THE EXCISED MAMMALIAN HEART BY PERFUSION WITH OIL.

BY TORALD SOLLMANN.

(WITH THE COLLABORATION OF E. D. BROWN AND W. W. WILLIAMS.)

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MAGNUS<sup>1</sup> in 1902 published some experiments showing the persistence of the contractions of the excised heart for one hour when the coronary vessels were perfused under pressure with oxygen gas and for one-half hour with hydrogen. The contractions ceased promptly on the perfusion of carbon dioxide. Magnus seeks to explain the persistence of the contraction during the perfusion of indifferent gases by the removal of deleterious gaseous waste products.

This explanation did not appear quite satisfactory to me; it seemed likely that the pressure used in the perfusion might be the essential factor. To determine this point, we undertook the perfusion of excised dogs' and rabbits' hearts with indifferent fluids, viz., cottonseed and paraffin oil (liquid petrolatum). The perfusions were made with a simplified Langendorff arrangement (*i. e.*, with empty chambers), with a pressure of 1.2 to 2 metres of oil, and temperatures varying between 30° and 42° C. The coronary circulation was generally first flushed with Locke's solution, to prevent the possibility of clotting. The perfusion of the oil was started only after the heart beat had ceased. The two oils and the hearts of dogs and rabbits gave identical results, as follows:

1. The perfusion of the oil starts the heart to beating rhythmically and forcibly; the contractions persist for about half an hour or longer. The two sides of the heart generally contract simultaneously and regularly, but irregularities are sometimes observed. Fibrillary contraction was never noted. The left ventricle stops before the right.

<sup>1</sup> MAGNUS, R.: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlvii, p. 200.



The perfusion of metallic mercury<sup>1</sup> also shows some contractions, especially of the right heart; but the vitality seems to be lost very soon. The muscle dries very rapidly when perfused with dry mercury.

The oil perfusion did not succeed in reviving hearts which had been excised from the body for some time. After the contractions are started they may persist for a few minutes when the pressure is removed.

2. The contractions are not due to the heat, for they are never seen when the heart is filled and laid in the warmed oil without being perfused.

3. To determine whether the effect of the oil is due to distention of the vessels, or to flow through the coronary arteries, a rabbit's heart was excised together with the lungs, and all the tissues exclusive of the aorta were included in the mass ligature. This left the coronary arteries open, but prevented all outflow from the right heart, and consequently from the coronary veins. The heart thus prepared beat when the oil was admitted into the coronary artery.

This experiment disposes of Magnus's explanation, the removal of waste products.

It would seem, therefore, that the essential cause of the resumption of the heart beat, in the mammalian heart, is the induction of an adequate pressure in the coronary vessels. I would not deny the importance of ions, oxygen, glucose, etc., for the prolonged functioning of the heart, or for its revival under unfavorable conditions,—this may be seen in our experiments; but these would appear to be mere accessory, sustaining factors, and not the essential cause, of the mammalian heart beat. I am also aware that the conditions must be different in the hearts of frogs, turtles, etc., which beat in solutions without pressure. It may further be true that there are other conditions besides pressure which may cause the mammalian heart to beat.

It seems to me that the above results are only explainable on the assumption that the mammalian heart beat is the consequence of a stimulus initiated by the distention of the coronary vessels under pressure.

It may be remarked that in so far as such a stimulation can be conceived more readily as originating in nervous than in muscular

<sup>1</sup> This was suggested to me by Prof. L. LOEB.

structures, these experiments favor the neurogenic theory of the origin of the mammalian cardiac contraction.

Protocols of the principal experiments are appended. (The dogs were killed by hemorrhage, the rabbits by the medulla stroke.)

PLAIN PERFUSION WITH PARAFFIN OIL.

*Experiment 5.*— Dog. 9.26. Heart excised.

9.39½. Oil admitted; pressure<sup>1</sup> 5 feet, temperature 32° C.<sup>2</sup>

9.41. Slight beat of right ventricle; temperature 34°.

9.42. Pressure increased to 6½ feet.

9.48½. Entire heart beats strongly, the contractions occur in groups of five, with pauses between, 20 per minute; temperature 36°.

9.52. Beats have become regular, 18 per minute.

9.54. The right ventricle beats stronger, the left very faintly; rate 36, temperature 37.5°.

9.55¼. Pressure reduced to 5¼ feet.

9.56. Regular; rate 30, temperature 35°. The pressure is taken off for one minute.

9.57. Regular; rate 29, temperature 37°.

9.58¼. Pressure raised to 6½ feet.

10.00. Regular; rate 20, temperature 36°.

10.04¼. Regular; rate 29, temperature 37.5°.

10.06. Regular; rate 30, temperature 38°, weaker; stream of oxygen through oil.

10.08½. Only occasional beat. Practical standstill.

10.09½. Final standstill.

Time from excision of heart to final standstill is 43 minutes.

Time during which the heart contracted, with oil perfusion, is 28 minutes.

*Experiment 6.*— Dog. 10.25. Heart excised.

10.31. Oil admitted, pressure 6½ feet.

10.31½. Right ventricle contracts; temperature 39°.

10.33. Left ventricle also contracts, alternating with the right (the left ventricle always contracting just before the right).

10.34½. Rate 33, temperature 39°.

10.36–10.38¼. Pressure removed, the heart contracts only at intervals.

10.41. Regular, strong contractions resumed, both sides synchronous; rate 66, temperature 42°.

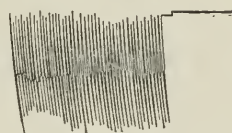


FIGURE 1.— Myocardiogram from dog, Experiment 6. Excised heart perfused with paraffin oil. The tracing illustrates the regularity of the contractions and the sudden stoppage at 10:44. Down stroke = systole.

<sup>1</sup> Height of perfusion flask above heart.

<sup>2</sup> The temperature of oil is taken in the cannula just before it enters the aorta.

- 10.44. *Contractions ceased suddenly*; temperature  $44^{\circ}$  (tracing 1).<sup>1</sup>  
 10.45½. Pressure lowered to  $5\frac{1}{4}$  feet.  
 10.49. Contractions have not resumed. *Locke's solution* and defibrinated blood started.  
 10.49½. Contractions resume in right heart; temperature  $41^{\circ}$ .  
 10.51. Both auricles and right ventricle contract; rate 51, temperature  $39.5^{\circ}$ .  
 10.52. Right auricle beats faintly, left ventricle in rigor; temperature  $38^{\circ}$ ; *changed to oil*.  
 10.58. Right auricle and ventricle beat regularly, the ventricle faintly; rate 36, temperature  $31^{\circ}$ .  
 11.01. Ventricle has stopped, auricle scarcely perceptible, but regular; rate 30, temperature  $30^{\circ}$ ; the contractions gradually become slower.  
 11.30. Rate 2.  
 11.35. No contractions.  
 Time from excision of heart to final standstill is 65 minutes.  
 Time during which the heart contracted during oil perfusion is  $12\frac{1}{2}$  minutes for the first period and 38 minutes for the second period.

## PLAIN PERFUSION WITH COTTON-SEED OIL.

- Experiment 3.* — Dog. 11.55. Heart excised.  
 12.03. Oil started, pressure 4 feet.  
 12.05. Faint beat in right auricle.  
 12.05½. Right ventricle contracts.  
 12.06. Both auricles and right ventricle beating; rate 15.  
 12.20. Still beating, but slower and weaker.  
 12.22. No beats, pinching of right ventricle causes a few additional beats.  
 12.28. No response.  
 Time from excision to standstill is 25 minutes.  
 Time during which the heart contracted, with oil perfusion, is 15 minutes.

## PERFUSION WITH MERCURY.

- Experiment 2.* — Rabbit. 11.11. Animal killed.  
 11.18. Heart excised.  
 11.20½. Mercury admitted; pressure 160 mm., temperature  $40^{\circ}$ .  
 11.21. Auricles beating.  
 11.25. *Mercury stopped*.  
 11.28½. *Contractions have ceased entirely*.  
 11.30. *Mercury perfusion resumed*.  
 11.30½. *Auricles beating again*.

<sup>1</sup> This was the only experiment in which the sudden stoppage occurred.

11.31½. Rate 60.

11.34. Pulsations cease, heart is very dry, aorta parchment-like.

11.38¼. Paraffin oil started; temperature 35°; no pulsations.

Time from excision to final standstill is 16 minutes. Time during which the heart beats with the perfusion of mercury is 4 minutes in the first period and three minutes in the second period. It is very apparent that the mercury starts the contractions.

*Experiment 1.* — Dog. 12. Heart excised.

12.05. Mercury perfused, pressure 150 mm; the auricles execute about 25 or 30 weak, wavy, circular contractions, then stop.

#### INTERMITTENT PERFUSION.

(See rabbit, Experiment 2, above).

*Experiment 7.* — Dog. (*Paraffin oil.*)

9.45. Heart excised.

9.59. Vessels flushed with oil; turned off at 10.

10.03. Oil turned on, pressure 5 feet.

10.04. Auricles beating.

10.05¼. Beats cease; temperature 45°.

Rare contractions of all chambers at intervals.

10.09½. Pressure raised to 6½ feet.

10.10¼. Contractions resumed, all chambers regular, but right heart stronger than left.

10.10¾. Rate 24, temperature 43°.

10.13. Rate 52, temperature 46.5°.

10.15¼. Rate 68, temperature 48°.

10.15¾. *Perfusion stopped.*

10.17. Contractions stop for about 20 seconds, then a group of five or six beats, again a pause of 15 seconds, then a few beats, then stoppage.

10.19. *Perfusion with oil resumed*; temperature 36°.

10.22¼. First contraction, right auricle.

10.22¾. Right auricle and right ventricle contracting; rate 16, temperature 34.5°.

10.24½. Same parts contracting; rate 12, temperature 34.5°.

10.25. Same parts contracting; rate 8, temperature 36°.

10.26¾. The right ventricle alone contracts.

10.29. The right ventricle alone contracts; rate 2, temperature 35°.

10.36. Nine beats in last 7 minutes; temperature 34.5°.

10.37. Contractions stopped; temperature 41°.

10.41. No contractions can be excited by mechanical stimulation.

Time from excision to final standstill is 50 minutes. Time during which heart contracted with oil perfusion is 11 minutes in the first period and 14 minutes in the second period.

## PERFUSION WITH VEINS TIED.

*Experiment 5.*—Rabbit. 11.07. Heart excised, flushed with Locke's solution, disconnected all vessels except aorta, tied with a mass ligature.

11.15. Immersed in paraffin oil at 32°.

11.19. Contractions have practically ceased, except some weak movements of the right ventricle.

11.19 $\frac{1}{4}$ . *Paraffin oil connected*, pressure 6 feet, left ventricle starts to beat.

11.19 $\frac{1}{2}$ . *Pressure removed.*

11.22 $\frac{1}{4}$ . Left ventricle ceases to beat.

11.22 $\frac{3}{4}$ . *Pressure resumed.*

11.23 $\frac{1}{4}$ . Both ventricles contract.

11.23 $\frac{1}{2}$ . Beating strongly and regularly; temperature 32°.

11.24 $\frac{3}{4}$ . *Veins opened.*

11.26. Still beating, strong and regular.

11.28 $\frac{1}{2}$ . *Perfusion stopped.*

11.31. Still beating.

11.33. Still beating, but very weakly.

11.34. Cotton-seed oil perfused, temperature 30°; contractions are strengthened at once.

11.44. Both sides still contracting, though very feebly; rate 24, temperature 30°.

11.48. Only the right auricle contracts.

11.53. Practically stopped.

The time from excision to final standstill is 46 minutes.

## WARMING WITHOUT PERFUSION DOES NOT START THE HEART.

*Experiment 8.*—Dog. 9.00. Heart excised.

9.10. Vessels flushed with normal saline.

9.30. Heart disconnected and immersed in paraffin oil at 35°.

10.00. There have not been any contractions.

*Experiment 4.*—Rabbit, in which the heart was placed directly in the oil, gave a similar negative result.

## NO BEAT CAN BE OBTAINED IF THE HEART IS PERFUSED SOME TIME AFTER EXCISION.

The heart of Dog, Experiment 8, just quoted, could not be revived by oil perfusion 70 minutes after excision. Oxygenated Locke's fluid, however, was also ineffective.

COMPARATIVE PHYSIOLOGY OF THE INVERTEBRATE  
HEART.—IV. THE PHYSIOLOGY OF THE CARDIAC  
NERVES IN THE ARTHROPODS.

A. J. CARLSON.

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I. THE DECAPOD CRUSTACEANS.

IT was pointed out in my paper on the anatomy of the cardiac nerves in the invertebrates that considerable discrepancy exists between the accounts of the innervation of the decapod heart as given by the different observers.<sup>1</sup> Those who have approached the question from the physiological side all agree that the heart is supplied with both augmentor and inhibitory nerves, but disagree as to the exact course and connections of these nerves. Plateau holds that the augmentor nerves pass from the cerebral ganglion to the heart along the ophthalmic artery, while the inhibitory nerves are given off from the thoracic ganglion and reach the heart along the sternal artery.<sup>2</sup> Jolyet and Viallanes locate both the augmentor and the inhibitory centres in the thoracic ganglion, but they do not trace the course of the fibres from this ganglion to the heart.<sup>3</sup> Conant and Clarke confirm the results of Jolyet and Viallanes, and they were in addition able to trace the course of the nerves from the thoracic ganglion to the heart.<sup>4</sup> The inhibitory fibres pass to the heart along with, or rather in the trunk of, the recurrent cutaneous pair of nerves which leave the ganglion dorsal to the point of origin of the nerves to the third maxillipeds. The augmentor nerves reach the heart along two pairs of small nerves that take their origin on the dorsal side of the thoracic ganglion near the origin of the nerves to the third maxillipeds

<sup>1</sup> CARLSON: Biological bulletin, 1905, viii, p. 123.

<sup>2</sup> PLATEAU: Bulletin de l'académie royale de Belgique, 1878, xlvi.

<sup>3</sup> JOLYET and VIALLANES: Annales des sciences naturelles, 1892, xiv, p. 387.

<sup>4</sup> CONANT and CLARKE: Journal of experimental medicine, 1896, i, p. 341.



and the first ambulatory appendages. Bottazzi confirms the results of Jolyet and Viallanes and of Conant and Clarke in placing the

origin of both the augmentor and the inhibitory nerves in the thoracic ganglion, the inhibitory centre being located anterior to the augmentor.<sup>1</sup>

The experiments of these observers (Plateau excepted) were made on the crab. I have repeated the experiments which go to prove that the thoracic ganglion gives rise to both the augmentor and the inhibitory nerves. This has been done both on crab and crayfish material. It is uniformly true that stimulation of the cerebral ganglion has no effect on the heart after the cerebro-thoracic commissures have been severed. If these commissures are left intact, the usual effect of the stimulation of the brain is inhibition of the heart in diastole.

Augmentation of the rhythm is occasionally obtained. In no instance did I observe any influence of the brain on the heart, except *via* the cerebro-thoracic commissures and the thoracic ganglion.

In the work on the crayfish (Palinurus) I made use of the graphic method. The thoracic ganglion was exposed from the ventral side. A portion of the

dorsal carapace in the region of the heart was removed so as to expose the dorsal wall of the pericardial cavity. The preparation was then supported ventral side down, and an upright connected with

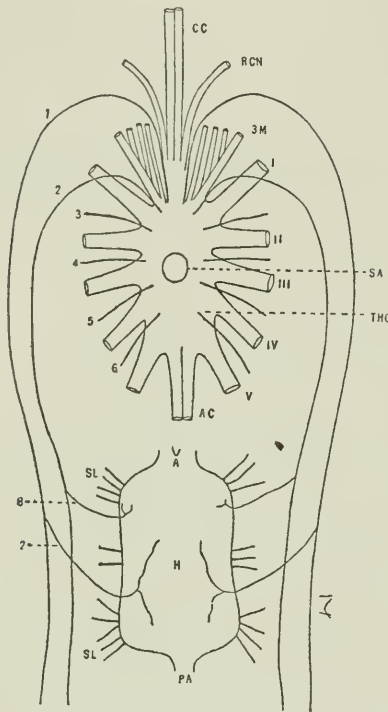


FIGURE 1.—Thoracic ganglion, heart and heart nerves of *Palinurus*, dorsal view, heart displaced posteriorly. *A*, anterior arteries; *AC*, abdominal commissures; *CC*, cerebro-thoracic commissures; *H*, heart; *PA*, posterior artery; *RCN*, recurrent cutaneous nerve; *SA*, sternal artery; *SL*, suspensory ligaments of the heart; *THG*, thoracic ganglion; *I-V*, nerves to corresponding ambulatory appendages; 1, 2, nerves to adductor muscles and heart; 3-6, nerves ramifying in the arterial plexus on the adductor muscles; 7, 8, cardiac nerves.

<sup>1</sup> BOTTAZZI: Centralblatt für Physiologie, 1901, xiv, p. 665.

the recording lever placed on the dorsal pericardium, or directly on the dorsal side of the heart, after removing the dorsal pericardium.

If the electrodes are placed on the anterior end of the thoracic ganglion, that is, near the origin of the cerebro-thoracic commissures (Fig. 1, *CC*), the stimulation nearly always produces complete or partial inhibition of the heart (Fig. 2, *A*). The cessation of the rhythm is accompanied by what appears to be a tonus relaxation. On the cessation of the stimulation the rhythm is usually augmented. When a relatively strong interrupted current is employed, augmentation instead of inhibition of the rhythm may be obtained, but this augmentation is frequently preceded by a brief inhibition (Fig. 2, *B*).

Stimulation of the recurrent cutaneous nerve (Fig. 1, *RCN*) in *Palinurus* has no effect on the heart, so far as I was able to determine. Stimulation of the pair of nerves that leave the dorsal side of the thoracic ganglion near the origin of the nerve to the third maxillipeds (Fig. 1, 1) produces partial or complete arrest of the heart in diastole (Fig. 3, *AB*), and stimulation of the pair of nerves leaving the ganglion near the origin of the nerves to the first ambulatory appendages (Fig. 1, 2), augments the rhythm (Fig. 3, *C*). These two pairs of nerves, therefore, contain the cardio-inhibitory and cardiac augmentor fibres respectively. The stimulation of the corresponding nerves given off near the second to the fifth ambulatory nerves respectively produced no effect on the heart as far as I could determine. For these experiments the greater part of the dorsal carapace was cut away, and the stomach, the liver, and portions of the reproductive gland removed from the body cavity. The nerves were isolated by removing the median flexor muscles. The preparation was supported ventral side down, and the upright connected with the lever rested directly on the heart. The stimulation of the inhibitory as well as the augmentor nerves after isolation frequently failed to influence the heart. This was in all probability due to injury to the nerves in the dissection rather than to individual variations in the specimens. The nerves of *Palinurus* die quickly after being isolated. Even in specimens in which the stimulation of the inhibitory nerves did not influence the rhythm, stimulation of the postero-lateral suspensory ligaments, along which the nerve fibres enter the heart, arrests the rhythm, as has been described by Dogiel for the crab.<sup>1</sup> In fact the inhibition of the heart is nearly always

<sup>1</sup> DOGIEL: *Archiv für mikroskopische Anatomie*, 1894, xliii, p. 223.

more complete, and may be maintained for a longer time by stimulating the inhibitory nerves in this region than by stimulating the thoracic ganglion or the inhibitory nerves in the thoracic cavity.

My results on the crayfish thus agree very closely with those of Conant and Clarke on the crab. *The heart of Palinurus is supplied with inhibitory and augmentor fibres from the thoracic ganglion. These fibres reach the heart in two separate pairs of nerves, the inhibitory fibres leaving the ganglion anterior to the augmentor fibres.*

## II. THE INSECTS AND THE ARACHNIDS.

To my knowledge no work has been done touching the physiology of the heart nerves in insects and arachnids, save that of Dogiel on the *Corethra* larva.<sup>1</sup> From the reactions of the heart of this larva to certain alkaloids Dogiel concluded that the heart was not provided with an inhibitory nervous mechanism. It must be admitted, however, that the evidence on which this conclusion is based is indirect and far from convincing. Dearborn<sup>2</sup> studied the influence of the central nervous system on the heart of the small crustacean *Daphnia*, reaching the conclusion that the heart of the *Daphnia* is provided with inhibitory but not with augmentor nerves.

My own work comprises the polyphemus moth, the grasshopper (*Dictyphorus reticulatus*), the tarantula (genus and species not known), and the horseshoe crab (*Limulus*). The horseshoe crab is now being classed with the arachnids, owing to its resemblance to the scorpions and the spiders rather than to the decapod crustaceans, to which group it was formerly referred. Some of the work on the heart nerves of *Limulus* has already been reported in this journal. But for the conclusive results obtained in *Limulus* and the orientation of the cardiac nervous mechanism afforded by that animal, little weight could have been accorded my results in the spider and the insects, as the small size of these animals render experiments on the heart and the heart nerves exceedingly difficult and uncertain. I found that the *Limulus* heart is connected with the central nervous system by a series of pairs of nerves, the inhibitory nerves coming off from the posterior end of the brain, the augmentor nerves being given off from the abdominal ganglia. The arrangement is thus similar to that in the crab and the crayfish (Fig. 1), the inhibitory heart

<sup>1</sup> DOGIEL: Mémoires de l'académie de St. Petersburg, 1877, xxiv, No. 10.

<sup>2</sup> DEARBORN: Medical news, 1903. Reprint, p. 20.

nerves leaving the central nervous system anterior to the augmentor nerves. This condition in the arthropods is the same as that found in the vertebrates. In *Limulus* it is, furthermore, evident that the intrinsic cardiac nervous complex is essential for the heart rhythm, the extirpation of the ganglion abolishing the rhythm at once and permanently. The nerve fibres passing from the ganglion to the heart muscle appear to be of ordinary motor type, but they exhibit very low excitability when tested by induction shocks.

In the tarantula, the polyphemus moth, and the grasshopper no nerves in the heart or connecting the heart with the central nervous system could be made out by microscopic methods. In the centipeds and the millepeds a nerve has been described on the median dorsal side of the heart, that is, in a position similar to that of the nerve cord or ganglion on the dorsal side of the heart of *Limulus*, but to my knowledge no connection between this nerve or nerve cord and the central nervous system has so far been made out.

Police describes in the scorpion a nervous connection between the heart and the supra-oesophageal ganglion or brain. Three pairs of "sympathetic nerves" issue from the brain to the viscera. One pair of these unites into one common trunk, which enters the heart. I do not have access to the papers of Police, but in the review of them in the *Zoologischer Jahresbericht* no mention is made of any nervous connection between the heart and the thoracic or abdominal ganglia.<sup>1</sup> Mr. McClendon,<sup>2</sup> of the Zoological Laboratory of the University of Pennsylvania, informs me that in the scorpion examined by him he also was able to make out the nerve cord on the dorsal side of the heart.

Owing to the microscopic size of the intrinsic heart nerves and their possible connections with the central nervous system, it appeared to be the only practicable way to determine the physiology of the cardiac nerves by studying the influence of the central nervous system on the heart rhythm. The hearts of the tarantula and the moth studied continue to beat rhythmically from ten to twenty minutes after being removed from the body. The heart of the large grasshopper studied will beat with perfect rhythm for two hours or more after removal from the body, if protected from evaporation. The intact heart of these animals, and the extirpated heart that is unin-

<sup>1</sup> POLICE: *Arch. zool. Napoli*, 1903, i, p. 179; *Zoologischer Jahresbericht*, 1903, p. 40.

<sup>2</sup> McCLENDON: *Biological bulletin*, 1904, viii, p. 38.

jured, beat simultaneously in all their parts, so far as can be made out by direct observation. To study the rhythm it thus suffices to remove a small portion of the chitinous epidermis dorsal to the heart in any one region. This can be done without injury to the pericardium or the heart. When the heart is thus exposed and the exposed brain stimulated with a weak interrupted current *the usual effect on the heart is augmentation of the rhythm.* This augmentation appears both in

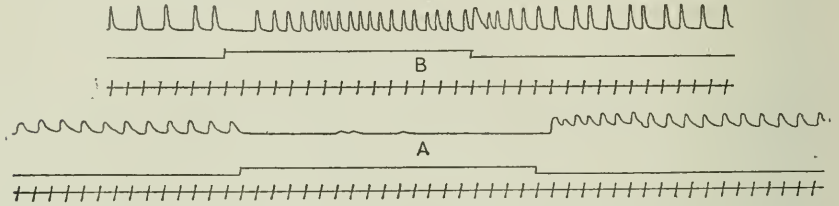


FIGURE 2. — Tracings from the heart of *Palinurus* on stimulation of the anterior end of the thoracic ganglion with the interrupted current. An upright placed on the dorsal side of the heart connects with the lever. *A*, inhibition, weak stimulus. *B*, inhibition and augmentation, strong stimulus. Time, seconds.

the rate and in the strength of the beats. It is particularly marked in the grasshopper. If the brain is similarly stimulated after severing all its connections, save the two large commissures connecting it with the thoracic ganglion, or if these cerebro-thoracic commissures themselves are stimulated, the effect on the heart rhythm is the same. It would therefore seem that the brain influences the heart only through its connections with the thoracic and the abdominal chain of ganglia. The heart nerves proper must then be given off by the latter ganglia, contrary to the findings of Police in the scorpion. In the grasshopper there is usually a latent period of several seconds between the beginning of the stimulation of the brain or the cerebro-thoracic commissures and the appearance of the augmentation of the heart. In the tarantula this was less marked. The heart rhythm is similarly augmented on stimulation of the thoracic ganglion. This ganglion is less easily exposed for this purpose, when the heart and its possible nervous connections are at the same time to be left intact. That applies all the more to the abdominal chain of ganglia, and I am therefore not able to state what nerves, if any, reach the heart from that source.

This stimulation of the brain, the cerebro-thoracic commissures, or the thoracic ganglion produces at the same time contractions of the



body muscles and respiratory movements. And the suggestion is obvious that the alteration in the heart rhythm may be due to these body movements altering the tension on or the pressure in the heart, etc., instead of to the influence of cardio-augmentor nerves. That the former is not the case can, I think, be shown quite conclusively in the grasshopper. If very great care is taken in dissecting away the chitenoid epidermis dorsal to the heart, the heart may be exposed to view throughout the whole length without severing the nervous connections between the heart and the ventral chain of ganglia. Such a preparation can be secured to a board by pins so that the movements of the body do not, or at least very slightly, affect the heart, yet on stimulation of the brain, the commissures, or the thoracic ganglion the augmentation of the heart rhythm is just as marked as in the experiments referred to above. This leaves little doubt but that *the heart of the tarantula, the polyphemus moth, and the grasshopper is provided with cardio-augmentor nerves, and that these, in the moth and the grasshopper, are given off, not by the brain, but by the thoracic, and possibly by the abdominal chain of ganglia.*

The hearts of these animals are in all probability also provided with inhibitory nerves from the same source. In the grasshoppers I frequently noticed that the heart stopped in diastole for periods covering three or four beats while the brain was being isolated for stimulation. In several specimens I noticed now and then similar brief periods of inhibition on stimulating the brain with very weak induction shocks. This inhibition was of more frequent occurrence on mechanical stimulation of the brain, such as picking it with the dissecting needle. The cessation of the rhythm had all the appearance of a true inhibition, such as the absence of a latent period or delay between the beginning of the stimulation and the cessation of the rhythm. In the polyphemus moth the results were less conclusive, but in the spider these inhibitory effects were even more marked than in the grasshopper. The conclusion seems therefore justified that *these arthropods are provided with both cardio-inhibitory and cardio-augmentor nerves.* The former nerves may be more powerful, so that when both sets of nervous mechanisms are stimulated at the same time, the effects of the inhibitory nerves are obscured. More definite knowledge must await the working out of the exact path of the nervous connections between the heart and the ventral chain of ganglia.

A further observation on the grasshopper's heart may be noted as touching the question of the cause of the heart rhythm. The heart



of the grasshopper extends almost throughout the whole length of the body. This long (4-5 cm.) heart beats simultaneously in all its parts, that is, so far as can be made out by direct observation, but the posterior two-thirds of the heart exhibits greater automatism than the anterior third of the heart. In fact, when the thoracic part of the heart is severed from the abdominal portion, the former usually exhibits no rhythm at all, while the posterior part continues to beat

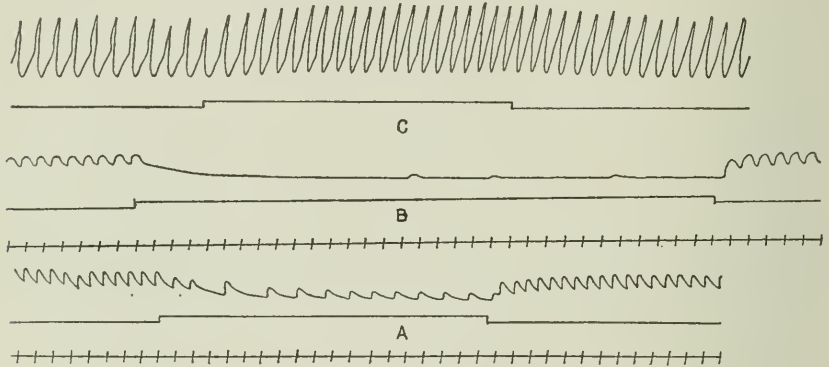


FIGURE 3. — Tracings from the heart of *Palinurus* on stimulation of the cardiac nerves (Fig. 1, 1 and 2) in the thoracic cavity. *A*, *B*, stimulation of the inhibitory nerve showing complete and incomplete inhibition of the rhythm. *C*, stimulation of the augmentor nerve (Fig. 1, 2). Time, seconds.

undisturbed. This is precisely the condition found in the heart of *Limulus*, and I have shown that in the heart of *Limulus* this difference is due, not to any difference in the character of the heart muscle, but to the difference in the distribution of the ganglion cells. The inference is natural that this peculiarity of the grasshopper's heart is due to the same cause, but the determination of this point must again await more detailed knowledge of the structure of the heart.

This paper concludes the present line of inquiry into the anatomy and physiology of the heart nerves in the invertebrates, an inquiry undertaken with the view of determining the nature of inhibition of the heart on direct stimulation with the induced current. The work has added to our knowledge of the physiology of the heart nerves both in the molluscan and the arthropod phyla, in the latter particularly through the results obtained in *Limulus*. It has led me to the conclusion that *the molluscan and the arthropod heart is without exception supplied with regulatory nerves, augmentor or inhibitory*—in most cases, and probably in all, with both. The work of Hunter, though not conclusive, points to similar conclusions regarding the tunicate

heart.<sup>1</sup> These types of cardiac nerves exhibit essentially the same physiological peculiarities as the corresponding nervous mechanism in the vertebrates. This is not saying that there are not gaps in the chain of evidence here presented to be filled in by further work. The heart nerves of the large scorpion should be studied. The heart nerves of the nudibranch molluscs should particularly be looked into further, touching the question of the time of development of the augmentor and the inhibitory cardiac nervous mechanisms.

H. E. Hering<sup>2</sup> has recently intimated that in my papers I do not consider whether or not the heart of invertebrates is comparable to the vertebrate heart either in structure, function, or conditions of activity. That criticism is rather surprising to me as, by citation of the literature and by my own observations, I have taken pains to point out that there appears to be no fundamental difference between the invertebrate and vertebrate heart. The study of the cardio-regulative nerves in the invertebrates supports this conclusion. There appears to be some difference in the physiological properties of the heart muscle, but I have a body of data, not yet published, which show that this difference is only one of degree, not of kind, and hence not fundamental. I have not taken into consideration to any great extent the minute histological structure of the heart tissues. I leave it to histologists to delineate fundamental differences in the muscular tissues and the nervous tissues of the vertebrate and the invertebrate heart.

Hering's criticism refers particularly to my papers on the heart of *Limulus*, stating that some of the observations on the *Limulus* heart do not agree with what is known of the physiology of the mammalian heart. As Hering does not point out specific instances, it is difficult for me to know to what observations he refers. The fundamental facts I have demonstrated in the heart of *Limulus*, namely, the neurogenic nature of the rhythm, the nervous nature of conduction and co-ordination, the point of action of the inhibitory nerves, the rate of conduction in the intra-cardiac nerve plexus, the nature of conduction in water rigor, — these fundamental demonstrations cannot be opposed to anything which has been demonstrated on the mammalian heart for the simple reason that these points have not been, and perhaps cannot be, put to a crucial test in the heart of a mammal.

<sup>1</sup> HUNTER: This journal, 1903, x, p. 1.

<sup>2</sup> HERING: Archiv für die gesammte Physiologie, 1905, cviii, p. 295.

FURTHER EVIDENCE OF THE DIRECT RELATION BETWEEN THE RATE OF CONDUCTION IN A MOTOR NERVE AND THE RAPIDITY OF CONTRACTION IN THE MUSCLE.

A. J. CARLSON.

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THE comparison of the rate of conduction of the impulse in the motor nerves of several molluscs and in the vagus and the hypoglossal nerves of the California hagfish with the rapidity of contraction in the muscles supplied by these respective nerves led me to the conclusion that "the rate of conduction in the motor nerves stands in direct relation to the rapidity of contraction of the muscle supplied by the nerve." That is, in any one animal the more rapidly contracting muscle is supplied with a more rapidly conducting nerve.<sup>1</sup>

The evidence establishing this principle as a general law must necessarily be cumulative in character. The greater the number of nerve-muscle mechanisms in which this relation has been shown to hold good, the stronger becomes the probability that it is true of all nerve-muscle mechanisms. If this relation between the rapidity of the physiological processes of conduction in the nerve and contraction in the muscle holds good in general, we ought to find a slow conduction in the nerves supplying the musculature of the viscera and the heart. The latter I have shown to be the case in *Limulus*.<sup>2</sup> In *Limulus* the intrinsic nervous plexus in the heart can be isolated for accurate measurement of the rate of conduction, so that actual comparisons may be made. These intrinsic heart nerves in *Limulus* conduct with a rate of only 40 cm. per second. The rate in the periph-

<sup>1</sup> CARLSON: This journal, 1904, vii, p. 401.

<sup>2</sup> CARLSON: This journal, 1906, xv, p. 101.

eral motor nerves is 325 cm. per second, or from eight to ten times swifter than in the heart nerves. We shall presently see that the contraction time of the heart muscle and the limb muscles exhibits a corresponding difference.

Finding the rate of conduction in the ambulacral nerves of *Limulus* only  $3\frac{1}{4}$  to  $3\frac{1}{2}$  m. per second, I decided to repeat Fredericq and Vandeveldé's measurements of the rate in the first ambulacral nerve of the lobster.<sup>1</sup> These observers concluded that the ambulacral nerves in that crustacean conduct at the rate of 6 m. per second, or almost twice as rapidly as the corresponding nerves in *Limulus*. I expected to find a similar difference in the contraction time of the muscle. The work was also extended to include the corresponding nerve-muscle mechanisms of the spider crab.

The measurement of the rate of conduction in the ambulacral nerve of these animals offers but little difficulty. I have described the nerve-muscle preparation and the experimental procedure in the paper dealing with the results in *Limulus*, and as the nerve-muscle preparation and the experimental procedure were the same for the lobster and the spider crab, no further description is needed in this connection. The reader is referred to that paper.

In the work on the lobster I first made use of a preparation from the second ambulacral appendage, but on finding the rate of conduction in this nerve twice as great as that recorded by Fredericq and Vandeveldé for the first ambulacral nerve of the same animal, I repeated the experiments, using a preparation from one of the pinchers.

Only fresh specimens in perfectly good condition were used. The motor nerves both of the lobster and the spider crab die soon after being isolated. The dissections must therefore be done rapidly. Only a few pairs of good records can be obtained from each preparation.

To illustrate the accuracy with which measurements of the conduction rate can be made on these preparations, one series of measurements is given in detail in Table I. The extremes of the twelve experiments summarized in Table II is 10 m. and 14.5 m. respectively, but the greater number falls between 11 and 12 m. per second. The twelve experiments recorded in this table were all made on preparations from the second ambulacral appendage. These measurements leave little doubt, that this nerve conducts at the rate

<sup>1</sup> FREDERICQ and VANDEVELDE: *Bulletin de l'académie royale de Belgique* 2 sér., xlvii.

of 12 m. per second. It seems possible that there might be an actual difference in the nerves of the first pair and those of the last four pairs of appendages. Fredericq and Vandeveldé found the rate in the first nerve to be only 6 m. per second. Accordingly I made measurements on four preparations from the first appendage, but as

TABLE I.

Detail measurements of experiment No. 2, Table II. Rate of conduction in the ambulacral nerves of the lobster (*Homarus*), June 18, 1905.

Total latent time in seconds.	
Distal.	Proximal.
0.025	0.017
0.025	0.017
0.025	0.016
0.026	0.018
Aver. 0.0252	0.017
Difference in latent periods, 0.008 second. Length of nerve, 10 cm. Rate of conduction, 12.50 m. per second.	

these give the values of 10 m., 11.6 m., and 9 m. respectively, I conclude that there is no such difference in these nerves. The low rate found by Fredericq and Vandeveldé was possibly due to poor condition of the material, as I found that preparations from specimens in poor condition might show as low a rate as 4 or 5 m. per second. Such preparations were excluded, only those from perfectly fresh specimens being included in Table II.

The contraction and relaxation of the adductor muscles of the forceps of the last four pairs of ambulacral appendages occupies about 0.25 second. A typical pair of records obtained in this series of experiments is reproduced in Fig. 1. The contraction time of the adductor of the chela is at least twice as great. The shortening of this muscle is just about as rapid as that of the adductor of the other limbs, but the relaxation is much slower. This difference

between these muscles led me to suspect a difference of rate of conduction in the nerves, but the measurements did not bear out this supposition.

TABLE II.

Summary of the measurements of the rate of conduction of the impulse in the ambulacral nerves (second pair) of the lobster (*Homarus*).

No. of experiment.	No. of pairs of records.	Length of the nerve in cm.	Transmission time in seconds.	Rate in m. per sec.
1	4	10.0	0.010	10.00
2	4	10.0	0.008	12.50
3	6	9.0	0.007	12.87
4	5	8.0	0.007	11.37
5	3	10.0	0.009	11.10
6	5	9.5	0.0075	12.66
7	3	8.5	0.0080	10.62
8	4	8.5	0.0060	14.11
9	3	10.0	0.0080	12.50
10	3	9.0	0.0080	11.25
11	4	8.0	0.0070	11.37
12	3	10.0	0.0090	11.10
Average rate, 11.75 m. per second.				

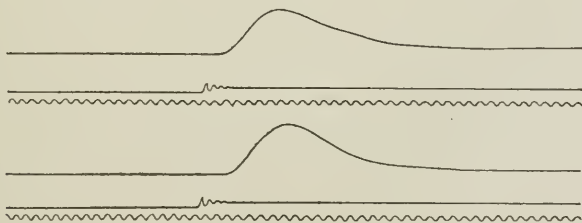


FIGURE 1.—Tracings of the contraction of the adductor of the forceps of the lobster (*Homarus*) on stimulation of the ambulacral nerve near the thoracic ganglion and near the forceps. Length of nerve,  $9\frac{1}{2}$  cm. Transmission time, 0.008 second. Rate of conduction, 12 m. per second. Time, 100 d. v. per second.



The work on the spider crab was rendered difficult by the exceedingly thick and calcareous carapace. The carapace of this crustacean contains so small an amount of organic constituents that it resembles the shell of the lamellibranchs or the marine gasteropods rather than the carapace of the lobster. This tough carapace renders dissection difficult and injuries to the nerve more liable.

TABLE III.

Detail measurements of experiment No. 4, Table IV. Rate of conduction of the impulse in the ambulacral nerves of the spider crab, June 24, 1905.

Total latent time in seconds.	
Distal.	Proximal.
0.063	0.048
0.064	0.050
0.070	0.053
Aver. 0.066	0.050
Difference in the latent time, 0.016 second. Length of nerve, 10 cm. Rate of conduction, 6.25 m. per second.	

The eight measurements summarized in Table IV give an average rate of about 5 m. per second, with a variation from 4 to 6 m. It is probable that the actual rate in perfectly healthy specimens is nearer 6 than 5 m. per second. The lower rate in experiments 1, 2, 3, and 6 is probably due to unavoidable injury to the nerves in dissection.

The contraction time of the adductor muscle of the forceps of the spider crab is about 0.50 second. The contraction time is twice as long and the rate of conduction twice as slow as in the corresponding muscle and nerve in the lobster. I expected some such difference between these two crustaceans, as the spider crab is very slow in its movements in comparison with the lobster. This sluggish activity of the locomotive tissues in the spider crab is probably to be associated with the presence of the very strong carapace or epidermis. This coat is invulnerable to many of the enemies of the crab, so that quick escape has not become essential.

TABLE IV.

Summary of the measurements of the rate of conduction in the ambulacral nerves of the spider crab.

No. of experiment.	No. of pairs of records.	Length of nerve in cm.	Transmission time in sec.	Rate in m. per sec.
1	4	10.0	0.023	4.30
2	3	10.0	0.026	4.00
3	6	11.0	0.021	4.73
4	3	10.0	0.016	6.25
5	2	11.0	0.018	6.10
6	5	12.0	0.025	4.80
7	5	10.0	0.020	5.00
8	4	10.5	0.017	6.30
Average rate, 5.15 m. per second.				

The corresponding muscle and nerve in these three crustaceans thus exhibit the following relation :

TABLE V.

	Contraction time (adductor of forceps).	Rate of conduction (ambulacral nerve).
Lobster . . . . .	0.25 sec.	12.00 m.
Spider crab . . . . .	0.50 "	6.00 "
Limulus . . . . .	1.00 "	3.25 "

In these nerve-muscle mechanisms the contraction time increases almost with mathematical exactness as the rate of conduction diminishes. A similar comparison between corresponding nerve-muscle preparations in gasteropod and cephalopod molluscs bring out the same relation :

TABLE VI.

	Contraction time (mantle muscle).	Rate of conduction (pallial nerve).
Squid . . . . .	0.20 sec.	4.50 m.
Octopus . . . . .	0.50 "	2.00 "

TABLE VII.

	Contraction time (foot muscle).	Rate of conduction (pedal nerve).
Limax . . . . .	4.0 sec.	1.25 m.
Pleurobranchæa . . . . .	10.0 "	0.75 "
Ariolimax . . . . .	20.0 "	0.40 "

An examination of Table VIII shows, however, that no such exact ratio between contraction time and conduction rate exists between different nerve mechanisms of different animals. Thus the mandibular nerve of the hagfish conducts much slower than the ambulacral nerve of the lobster, although the hagfish's retractor of the jaw con-

TABLE VIII.

Comparison between the contraction time of the muscle and the rate of propagation of the impulse in the nerve.

Species.	Muscle.		Nerve.	
	Muscle.	Contraction time in seconds.	Nerve.	Rate of the impulse in m.
Frog . . . . .	gastrocnemius	0.10	sciatic (medullated)	27.00
Snake . . . . .	hypoglossus	0.15	hypoglossus (medullated)	14.00+
Lobster . . . . . (Homarus)	adductor of forceps	0.25	ambulacral (non-medullated)	12.00
Spider crab . . . . .	adductor of forceps	0.50	ambulacral (non-medullated)	6.00
Hagfish . . . . .	retractor of jaw	0.18	mandibular (non-medullated)	4.50
Squid . . . . . (Loligo)	mantle (fin)	0.20	mantle nerve (non-medullated)	4.50
Limulus . . . . .	adductor of forceps	1.00	ambulacral (non-medullated)	3.25
Hagfish . . . . .	gill sac	0.45	vagus (non-medullated)	2.50
Octopus . . . . .	mantle	0.50	pallial (non-medullated)	2.00
Slug . . . . . (Limax)	foot	4.00	pedal (non-medullated)	1.25
Sea hare . . . . . (Pleurobranchæa)	foot	10.00	pedal (non-medullated)	0.75
Slug . . . . . (Ariolimax)	foot	20.00	pedal (non-medullated)	0.40
Limulus . . . . .	heart	2.25	nerve plexus in heart (non-medullated)	0.40

tracts more rapidly than the adductor of the lobster's forceps. The intrinsic heart nerves of *Limulus* and the pedal nerves of the slug (*ariolimax*) conduct at nearly the same rate, yet the foot muscle of this slug appears to contract and relax much slower than the heart muscle of *Limulus*. The actual contraction time of a muscle cell in a complicated muscular apparatus like the gasteropod foot is, however, difficult to determine, and it is possible that the more accurate determination of this element will reveal a closer agreement. The data in Tables IV, V, and VI, make it highly probable that the principle of *direct relation or ratio between contraction time and conduction rate holds good for corresponding nerve-muscle mechanisms of different groups of animals*. I expect that accurate measurements on the sciatic nerve of the tortoise and the toad will show slower conduction than in the sciatic of the frog. My results on the two nerve-muscle mechanisms of the hagfish and on the two nerve-muscle mechanisms of *Limulus* show further that *this principle obtains in the case of different nerve-muscle mechanisms of the same animal*. But the comparison of all the data at hand pertaining to this question (Table VII) shows that *the principle is not strictly applicable to different nerve-muscle mechanisms of all animals*.

## THE ACTION OF SALINE SOLUTIONS ON THE VITALITY OF BLOOD VESSELS.

By R. A. HATCHER.

[From the Department of *Materia Medica and Therapeutics*, Cornell University Medical College.]

THE present research was undertaken to determine the relative efficiency of Ringer's, Locke's, and normal saline solutions in prolonging the vitality of the blood vessels.

The composition of the Ringer's solution was as follows: Potassium chloride 0.2 gm., calcium chloride 0.2 gm., sodium bicarbonate 0.2 gm., sodium chloride 9 gms., and distilled water to make one litre. Locke's solution was the same except for the addition of one gram of glucose. The normal saline was made by dissolving 9 gms. of sodium chloride in one litre of distilled water.

Adrenalin was employed to show the persistence of vitality of the vessels, through its well-known property of causing constriction. Brodie and Dixon<sup>1</sup> found that adrenalin acts upon the nerve endings in blood vessels, and Elliott<sup>2</sup> concluded that the irritable substance at the myoneural junction, upon which adrenalin acts, depends for continuance of life upon the nucleoplasm of the muscle cell, not of the nerve cell. In addition to the action of adrenalin, the capacity for reducing hæmoglobin when blood is perfused through the vessels, was also noted as evidence of vitality.

The kidneys were the organs chosen for the experiments, because of their vascularity and the consequent readiness with which the adrenalin action may be shown with an oncometer and by variation of the venous flow, and because their active metabolism affords another convenient way of testing vitality by the reduction of hæmoglobin when blood is perfused through them.

Dogs were used in all the experiments. They were anesthetized

<sup>1</sup> BRODIE and DIXON: *Journal of physiology*, 1904, xxx, p. 476.

<sup>2</sup> ELLIOTT: *Journal of physiology*, 1905, xxxii, p. 400.

with morphine and a mixture of alcohol, chloroform, and ether. The general procedure for perfusion was that described by Sollmann.<sup>1</sup> The kidneys were excised, cannulas were placed in the ureters, renal veins, and arteries, and the arteries connected with reservoirs suspended at a height of ninety to one hundred and twenty-five centimetres, the kidneys were washed free of blood (by perfusion) and placed in oncometers of a simple type connected with water manometers graduated in centimetres.

The dogs were bled and the blood defibrinated for subsequent perfusion in order to note the reduction of hæmoglobin.

The first three experiments dealt with Ringer's solution and normal saline. They were made at room temperature, 25° C. The remaining experiments were with Locke's and normal saline solution (Ringer's in Experiment IX) and the kidneys were kept at 30° C to 38° C. Brodie and Dixon<sup>2</sup> found that a fall of several degrees in temperature made no difference in the reaction. I found no very great difference with an extreme variation of some 20° C. in different experiments.

#### DISCUSSION.

The results of the first three experiments show that Ringer's solution is much more effective than normal saline in maintaining the vitality of the vessels, as shown by the adrenalin reaction.

Adrenalin produced but little or no fall of the vein flow or oncometer after three hours of saline perfusion in Experiments I and II, and but little after two and a half hours in Experiment III; these results were confirmed in Experiments IV and V.

On the other hand the reaction was vigorous after three hours' perfusion with Ringer's in Experiments I and III, after nearly six hours in Experiment II, and after seven hours' perfusion in Experiment IX.

The intensity of the reaction to adrenalin appears to be largely independent of the concentration of the reagent, since no material difference could be seen between the effects of perfusing with 1-50,000 and simply injecting from one to two c.c. of 1-10,000 into the tube near the arterial cannula.

The kidneys perfused with normal saline solution in Experiments I and III showed but slight reduction of hæmoglobin after three hours'

<sup>1</sup> SOLLMANN: This journal, 1905, xiii, p. 241.

<sup>2</sup> BRODIE and DIXON: *Loc. cit.*



perfusion, whereas the kidneys perfused with Ringer's solution in Experiment III showed much greater reduction after three hours and fifteen minutes; this was also true after twenty-four hours' perfusion, with Ringer's solution in Experiment IX.

The kidneys perfused with Locke's solution gave the adrenalin reaction after more than twenty-four hours in Experiments V, VII, and IX, but not in VI.

The reduction of hæmoglobin was much greater after twenty-four hours' perfusion with Locke's solution in Experiments V, VIII, and IX, and after twenty hours in Experiment VII, and after eight and nineteen hours' perfusion with normal saline in Experiments V and VII respectively. In Experiment V there was little or no reduction after twenty-five hours' perfusion with normal saline solution, and reduction was scarcely perceptible after twenty-four hours' perfusion in Experiment VIII. This result in Experiment VIII was the more convincing because the vein flow of the Locke's solution was as high as eight drops per minute, and as low as four with the normal saline solution.

Repeated efforts to perfuse blood mixed with two per cent of sodium chloride were unsuccessful after twenty-four hours in Experiment VII, though 0.9 per cent sodium chloride was perfused without difficulty. A barely perceptible difference of hæmoglobin reduction in favor of normal saline was seen after twenty-five hours in Experiment VI — the one exception in the entire series of observations — it was probably due to some accident of perfusion.

In Experiment VII perfusion of blood after nineteen hours of normal saline resulted in but slight reduction of hæmoglobin, but the blood collected from the vein in a test tube was allowed to stand half an hour or longer, when it was found to be nearly black; upon shaking the tube a few moments, the color of the blood became bright red; upon standing, it rapidly changed as before, this was repeated several times. The same phenomenon was noticed in Experiment IX, after twenty-four hours' perfusion with Ringer's solution, but the change of color was not nearly so rapid.

Experiment IX was made for the sake of comparison between Ringer's and Locke's solutions at the same temperature upon the kidneys of the same dog. So far as conclusions can be drawn from a single experiment, Locke's is shown to be the more efficient in maintaining the reaction to adrenalin. This agrees with the results of the other experiments, in which, however, the temperature was

not the same and in which kidneys of the same dog were not compared.

In this experiment the temperature was kept more nearly uniform at 35° C. than in any of the others, and it would appear that the temperature is somewhat more favorable to maintaining the adrenalin reaction than is a lower one.

#### CONCLUSIONS.

The following conclusions apply to the dog's kidney :

1. Kidneys show little or no reaction to adrenalin after about three hours' perfusion with normal saline (0.9 per cent sodium chloride in distilled water) at about 25° C., and if the perfusion be continued for ten to twenty-four hours and blood be then substituted for the normal saline perfusion, only a slight reduction of hæmoglobin occurs.

2. Kidneys perfused with Ringer's solution (formula given on p. 144) show the adrenalin reaction for a somewhat longer time than those in which normal saline is used, and the reduction of hæmoglobin after three to six hours is somewhat greater than is the case after normal saline.

3. When Locke's solution is perfused the kidneys react to adrenalin after twenty to twenty-seven hours, and after twenty-four hours the reduction of hæmoglobin is usually distinctly greater than after normal saline for the same period.

## FURTHER EVIDENCE OF THE SIMILARITY BETWEEN CATALYSIS AND ENZYME ACTION.

By C. HUGH NEILSON.

[From the Physiological Department of St. Louis University.]

IT is a well-known fact that properties which were once thought characteristic of enzymatic action alone are also possessed by many substances in the inorganic world, — namely, colloidal solution of platinum, gold, silver, etc., platinum black, manganese dioxide, etc. Among these properties of enzymes are the following: the enzyme produces an amount of chemical change out of proportion to the enzyme used; the enzyme acts merely as a catalyzer; and many of these enzymes have a reversible action.

These facts apply equally well to certain metals and their oxides, such as the above-mentioned. There is a further similarity between the action of enzymes and these metals in that many of the changes brought about by enzymes can also be produced by these metals. Berzelius,<sup>1</sup> Thenard,<sup>2</sup> Schönbein,<sup>3</sup> and others, have shown the similarity between the catalysis of hydrogen peroxide by organic ferments and finely divided platinum. The inversion of cane sugar, which is brought about by many enzymes, can also be produced by finely divided platinum, palladium, etc., as shown by Rayman and Sulc.<sup>4</sup> Kastle and Loewenhardt<sup>5</sup> found that lipase hydrolyzes ethyl butyrate into butyric acid and ethyl alcohol, and also synthesizes it from these compounds. Neilson<sup>6</sup> obtained the same results in the action of platinum on ethyl butyrate.

<sup>1</sup> BERZELIUS: *Jahresberichte*, 1836, xiii, p. 237.

<sup>2</sup> THIENARD: *Mémoires de l'Académie des Sciences*, 1818, iii, p. 385.

<sup>3</sup> SCHÖNBEIN: *Journal für praktische Chemie*, 1, lxxxix, pp. 32 and 325.

<sup>4</sup> RAYMAN and SULC: *Zeitschrift für physikalische Chemie*, 1892, xxxi, p. 262. footnote.

<sup>5</sup> KASTLE and LOEWENHART: *Chemical news*, 1901, lxxxiii, pp. 2150–2155; also, *American chemical journal*, 1900, xxiv, p. 491.

<sup>6</sup> NEILSON: *This journal*, 1903, x, pp. 192–200.

The object of this paper is to show further evidence of the similarity between the action of enzymes and that of metals. For this purpose I used the glucosides, salicin and amygdalin. These, as is well known, are split up by the enzyme emulsin, — the former being split up into glucose and benzoic aldehyde; the latter, into glucose, saligenin or oxybenzylic alcohol, and hydrocyanic acid. It occurred to me to use platinum black instead of the enzyme, emulsin.

In carrying out these experiments the following method was used: the platinum black was weighed out in the desired amounts and placed in 200 c.c. Erlenmeyer flasks. One hundred c.c. of the glucoside solution, of the desired concentration, were placed in the flasks together with the platinum black. The flasks were tightly corked, placed in an incubator registering  $40^{\circ}$ – $42^{\circ}$  C. and kept for a definite length of time. The flasks were shaken at intervals, as the platinum black settles in a short time. A control experiment with the same amount and concentration of the glucosides was always made to show whether any splitting had spontaneously occurred, or whether splitting from any cause, such as bacteria, the reagents used, etc., had taken place. In no case was there any evidence of sugar in the control experiments. The sugar was tested for qualitatively, and determined quantitatively, by the Haines method, which is a modification of Pavy's method.

**Experiments with salicin and platinum black.** — In consulting these experiments, it is found that the amount of splitting in a given time reached a maximum, and then with increasing time the amount of splitting was not in proportion to the time, being materially less in the last twenty-four hours than it was in the preceding. This is due, no doubt, to the effect of the products of the splitting — especially the saligenin. It is more probably due, however, to the salicylic acid produced by the oxidation of the saligenin to salicylic acid by the platinum. It is well known that salicylic acid has a marked retarding action on enzymes, as shown by Kastle and Loewenhardt<sup>1</sup> in its action on lipase; and by Neilson<sup>2</sup> in its action on platinum black in the hydrolysis of ethyl butyrate.

The solution at the end of the experiment, when the quantitative determination of sugar was made, gave a pronounced violet color upon the addition of ferric chloride, instead of the blue which should be given by saligenin. The solution of the splitting products was

<sup>1</sup> KASTLE and LOEWENHART: *Loc. cit.*

<sup>2</sup> NEILSON: *Loc. cit.*

evaporated to dryness on the water bath. The residue was then dissolved in a small amount of distilled water, most of it going into solution. The solution was filtered, and the few crystals remaining were placed in distilled water at 10° C. They did not go into solution. On heating, they dissolved and gave a faint violet color on the addition of ferric chloride. This shows that the salicylic acid and possibly some salicylic aldehyde, had volatilized by the evaporation of the solution to dryness on the water bath.

	Concentration of solution.	Amount of platinum.	Time.	Amount of sugar.
	per cent	grams	hours	gram
Effect of time on the rate of splitting . . . . .	2	5	72	0.041
Effect of time on the rate of splitting . . . . .	2	5	96	0.051
Effect of time on the rate of splitting . . . . .	2	5	120	0.055
Effect of amount of platinum . . . . .	2	1	96	0.015
Effect of amount of platinum . . . . .	2	5	96	0.059
Effect of concentration of solution . . . . .	1	3	96	0.021
Effect of concentration of solution . . . . .	2	3	96	0.028

Another fact which shows that the acid had volatilized is that the original solution, which gave a pronounced violet reaction with ferric chloride after it had evaporated to about one-half of its original volume, gave a faint violet color, or no reaction at all, on the addition of ferric chloride. The experiment also shows that the amount of splitting is approximately proportional to the amount of platinum used. Amounts of platinum were used varying from 200 mgs. up to 5 gms. and the amount of splitting was always proportional to the amount of platinum used. It is also seen that the concentration of the solution had practically no effect in the concentrations of 1 per cent and 2 per cent which were used.

**Experiments on salicin with manganese dioxide.**—The manganese dioxide used was thoroughly washed with distilled water until the wash water gave no test for acid. This was then dried in the drying oven. Varying amounts of this were added to 2 per cent salicin, and the amount of sugar determined qualitatively and quantitatively at

the end of a given time. The results agree with those brought about with platinum, with the exception that manganese dioxide does not produce as rapid splitting as the platinum, and that the splitting products do not have so powerful an action on stopping the action of the manganese dioxide as they do on the platinum.

**Experiments with amygdalin and platinum black.**—These experiments were carried out in the same manner as with salicin. It was anticipated, however, that the amount of splitting by the action of the platinum black on the amygdalin would be small, as it is well known, from Bredig's<sup>1</sup> work on the catalysis of hydrogen peroxide or colloidal platinum, and from Neilson's<sup>2</sup> work on the hydrolysis of ethyl butyrate by platinum black, that hydrocyanic acid has a marked retarding action on the catalytic action of platinum.

One of the splitting products of amygdalin is hydrocyanic acid; and according to the above experiments one would expect the action of platinum black on amygdalin to be retarded, if not stopped entirely. Such, indeed, was found to be the case, as the first experiment with tightly corked flasks was entirely negative. It occurred to me that by leaving the flasks uncorked the hydrocyanic acid would volatilize and the action would then proceed. This is in keeping with the well-known fact that removing one of the products of a chemical reaction allows the action to go on to completion.

By leaving the flasks uncorked it was found by a qualitative test that the platinum splits up the amygdalin. The amount of splitting, however, was small, as shown by the quantitative determination of the sugar. It was further seen that the action did not go on to completion, as the amount of sugar produced was small. The reason for this fact is obscure. Possibly the hydrocyanic acid did not completely volatilize. At no time could the odor of hydrocyanic acid be definitely recognized. This was probably due in part to the exceedingly small amount which was being produced at a given time, and in part to the peculiar odor which was given off from the mixture of amygdalin and platinum black,—an odor similar to that produced by a mixture of water and powdered zinc. The odor of benzoic aldehyde was also present after the solution had been slightly warmed. Possibly some of the hydrocyanic acid may have combined with benzoic aldehyde, one of the products of the splitting of amygdalin, and cyano benzene formed, and this may have exerted a retarding action on the

<sup>1</sup> BREDIG: *Anorganische Fermente*, 1900, p. 68.

<sup>2</sup> NEILSON: *Loc. cit.*



platinum. The amount of sugar produced was small, but gradually increased to a maximum, and then no more was produced. The effect of time, concentration of the amygdalin, and amount of platinum were similar results to those observed in the action of platinum black on salicin.

We thus see from these experiments that the similarity between the action of enzymes and the action of metals, which act merely as catalyzers, is carried still further. On the basis of these experiments and those of many other investigators we may say that enzymatic action is a catalysis and perhaps nothing more.

## FURTHER STUDIES ON THE PHYSIOLOGY OF HEART-BLOCK IN MAMMALS.<sup>1</sup>

By JOSEPH ERLANGER AND ARTHUR D. HIRSCHFELDER.

[From the *Physiological Laboratory of the Johns Hopkins University.*]

### INTRODUCTION.

**SUMMARY** of earlier experiments.<sup>2</sup>—In previous publications one of the authors has given the results of experiments upon the effect of gradually compressing the auriculo-ventricular bundle of His in the dog. The main results of these investigations may here be briefly summarized as follows:

1. Compression of the tissue in the region of the auriculo-ventricular bundle of His alone will block the passage from the auricles to the ventricles of the impulse which normally causes the heart to beat.
2. As no nerve trunks have been found in this region, conduction in the dog's heart is probably muscular.
3. By carefully grading the pressure on the auriculo-ventricular bundle, every stage of heart-block may be obtained; for example, 2:1, 3:1, etc. rhythms.
4. When the block is complete, the ventricles cannot be inhibited through the vagi, or but minimal slowing can be obtained under such circumstances.
5. It was also stated that when the block is partial, vagus inhibition of the ventricles may be obtained at least as easily as when the conductivity at the auriculo-ventricular junction is normal. But it should be mentioned here that further studies have shown that the duration of ventricular inhibition varies inversely as the degree of block when the vagi are stimulated with maximal tetanizing currents.
6. The accelerator nerves continue to exert their normal influence over the ventricles after the establishment of complete block.

<sup>1</sup> A preliminary notice of the results herein recorded appeared in the *Zentralblatt für Physiologie*, 1905, xix, p. 270.

<sup>2</sup> ERLANGER: *Zentralblatt für Physiologie*, 1905, xix, p. 9; to appear in the *Journal of experimental medicine*.

7. Finally, it was found that occasionally, at the moment the block becomes complete, the ventricular rate suddenly becomes very slow. Indeed, the ventricles practically stop beating, and after a longer or shorter period of standstill, their rate gradually increases until it becomes that which is usual in complete heart-block. The cause of this phenomenon was not determined, but the possibilities were enumerated and discussed, and reference was made to the possible relation of this phenomenon to the question of the action of the vagi on the ventricles.

**Object of present experiments.** — The experiments which form the basis of this communication were undertaken with the object primarily of investigating this phenomenon of *stoppage of the ventricles*. For it was thought that an understanding of it might shed some light, not only upon the phenomenon of cardiac inhibition, but also upon the causation of the syncopal attacks of Stokes-Adams disease during which the rate of the ventricles alone is markedly reduced.<sup>1</sup>

While these studies were in progress the opportunity was afforded of confirming, and of adding to, the observations on heart-block which have already been published. Reference will be made to some of these in the body of this paper; but for the confirmation of the main points the reader is referred to the protocols of the new experiments which form the basis of this communication.

#### GENERAL METHODS.

The method employed for producing heart-block was the same as that published in the earlier communications, namely: An L-shaped hook is introduced into the left ventricle through the wall of the aorta near its root and then turned in the left ventricle so as to perforate the septum ventriculorum just below the membranous septum. A metal block is then placed upon the protruding arm of the L, and by means of a nut tightened down so as to compress the auriculo-ventricular bundle against the horizontal arm of the L. In this way the auriculo-ventricular bundle, plus a variable bit of tissue in its vicinity, can be subjected to varying degrees of compression.

**Material.** — In all twenty-one new experiments have been performed.<sup>2</sup> Dogs were used in nineteen of these; a rabbit and a cat

<sup>1</sup> ERLANGER: *Journal of experimental medicine*, 1905, vii, p. 676.

<sup>2</sup> It will frequently be necessary to refer to the earlier experiments on heart-block which will be published in the *Journal of experimental medicine*. In order

in one each. All of the animals were anæsthetized with ether, the dogs receiving morphine in addition.

**Methods of recording.**—Simultaneous tracings were made of the movements of the right auricle, of the right ventricle, and of the time in fifths of seconds. At first the contractions of both the auricle and of the ventricle were recorded by means of tambours. But in the later experiments the ventricular movements were recorded with the myocardiograph of Roy and Adami. In the tracings the contractions are marked by down strokes.

**Autopsies.**—An autopsy was performed on every animal. These have shown that heart-block was obtained only, and then invariably, when the auriculo-ventricular bundle had presumably been included in the grasp of the clamp. Heart-block was obtained in all cases, but frequently not until several attempts had been made. The positions occupied by the clamp in the unsuccessful attempts were usually marked by hæmorrhages into the myocardium where it had been perforated and compressed. It was always evident that in these positions of the clamp the auriculo-ventricular bundle had not been compressed.

## I. ON THE RHYTHM OF DEVELOPMENT OF THE MAMMALIAN VENTRICLES AND ITS RELATION TO THE QUESTION OF THE ACTION OF THE VAGUS NERVE ON THE MAMMALIAN VENTRICLES.

### THE PHENOMENON OF STOPPAGE OF THE VENTRICLES.

**Description.**—The phenomenon, which we shall for present convenience term “stoppage of the ventricles,” may again be described as follows: While tightening the heart-clamp, the ventricles may come to a standstill more or less abruptly, whereas the auricles continue to beat with practically unaltered rate and rhythm. During this stoppage the ventricles are completely relaxed, and they are gradually distended by the rhythmic injection of blood into them by the contractions of the auricles. After a variable period of time the ventricles will contract and empty themselves. Their contractions then, to obviate confusion the experiments herein recorded will be designated ‘New Series.’ In the text the Roman numeral following the abbreviation N. S. refers to the experiment, and the Arabic numeral following it, to the procedure in that experiment which illustrates the point in question.

as a rule, gradually increase in rate until the slow constant rate of the ventricles in complete block obtains.

There seem to be two types of stoppage of the ventricles. In what is perhaps the more common type, the first ventricular cycle (by this is meant the time intervening between the ventricular contraction in question and the beginning of the next following ventricular contraction) is the longest. In the other type the successive ventricular cycles increase in length through 1, 2, 3, or rarely more, contractions. It is often difficult to be certain that the early contractions seen in the latter type of stoppage are not extra contractions excited by the manipulation of the clamp. But in some experiments (in this connection see N. S. XVI) this factor may be definitely excluded, because of the regular form of the tracings and by the fact that the same type is repeatedly obtained in the same experiment. Examples of the two types of stoppage are reproduced in Figs. 1 and 2.

**The relation of stoppage to the establishment of complete heart-block.** — The following fact is of great significance in determining the cause of stoppage of the ventricles. It can be seen, in those instances in which the changes in rhythm are not too sudden, that the onset of stoppage of the ventricles is invariably synchronous with the establishment of complete heart-block.

The facts which justify this conclusion follow:

*a.* During the phenomenon of stoppage the ventricular contractions are totally independent of the auricular contractions.

*b.* Often stoppage is preceded by shorter or longer stages of partial block (see Fig. 1). However, it not infrequently develops out of a 1:1 rhythm directly (see Table I).

*c.* Stoppage is almost invariably succeeded by a period of complete block in which the rate of the ventricles is perfectly constant. In some experiments the heart has never recovered from the complete block which followed stoppage (see Table I).

*d.* But it is the rule for the normal sequence to be slowly restored through stages of 3:1 and 2:1 rhythms (see Fig. 1). These stages may be quite protracted. However, occasionally the complete block which follows stoppage may give way to the normal sequence at once, *i. e.*, without passing through any of the intermediate stages of partial block (see Fig. 3). It could be seen that this mode of recovery occurred only in experiments in which complete block was obtained by obviously slight manipulation of the clamp. Thus, it

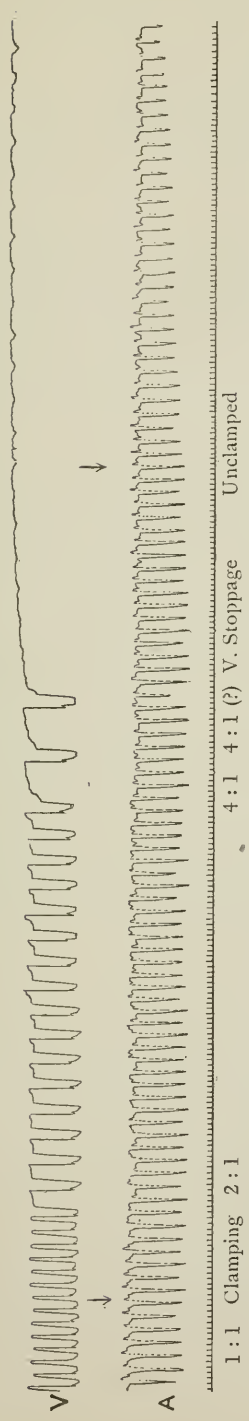


FIGURE 1.— One-half the original size. Effect of tightening and loosening the clamp on the auriculo-ventricular bundle. Stoppage of the ventricles of the first type. N. S. V, 2. In this and the succeeding figures the upper curves give the ventricular movements recorded with the myocardiograph, the lower curves the auricular movements recorded with tambours. Down strokes represent contractions. The time equals fifths of seconds.

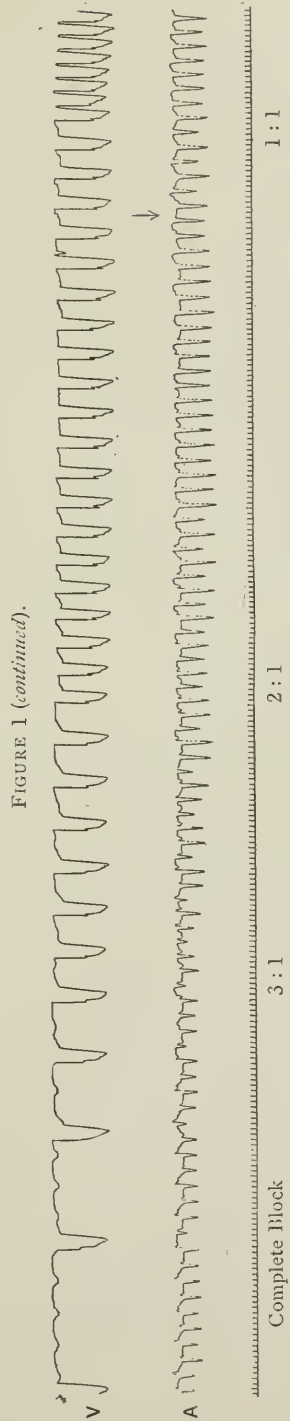


FIGURE 1 (continued).

Complete Block



occurred only, and then rather rarely, after stoppages which had developed out of a 1:1 rhythm directly.

It should be stated here that, as a rule, the clamp was opened as soon as evidence was obtained that stoppage had come on. It is

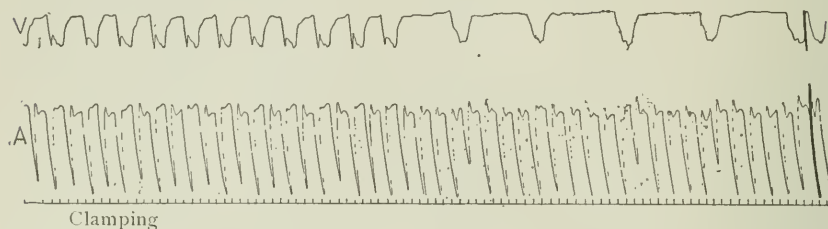


FIGURE 2. — Same. About four-fifths the original size. Stoppage of the second type developing from a 2:1 rhythm. N. S. XVI, 10.

possible that some of those instances of stoppage, in which the complete block disappeared before the ventricular rate had become constant, did not last as long as they might have had the compression not been removed (see Fig. 3).

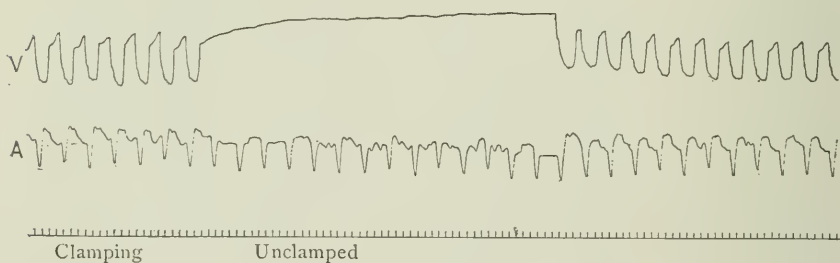


FIGURE 3. — About three-fifths the original size. Stoppage of the ventricles developing from a 1:1 rhythm and returning to a 1:1 rhythm without passing through intermediate stages of block. N. S. V, 16.

It seems probable that the mode of recovery from stoppage and from the complete block which usually follows stoppage is determined by the severity of the injury done to the auriculo-ventricular bundle while clamping (see XXVIII and XXXI).

The duration of the longest ventricular cycle of stoppage seems to depend upon a number of factors. Among these the following may be mentioned:

1. The stage of partial block and the duration of such stage at the moment the block becomes complete. In the accompanying table (No. I) it may be seen that, as a rule, stoppage of the ventricles is

longest when complete block is suddenly established while the rhythm is 1 : 1. But occasionally stoppage is not obtained even under these circumstances (*e. g.*, N. S. II and XII). And again prolonged stoppage has been obtained after protracted periods of a 2 : 1 rhythm (*e. g.*, N. S. V, 2, and XVI, 10). Only very short stoppages have been obtained after even brief stages of a 3 : 1 rhythm.

2. It seems that in the type of stoppage in which the ventricular cycles gradually increase in length, these cycles are not so long as in the other type. This is well illustrated in N. S. V (see protocol). Thus those stoppages in which the first ventricular cycle is the longest (Nos. 2 and 3) are of much greater duration than those that show ventricular cycles of increasing duration (9, 17, and 18).

3. Finally, it should be stated that there are variations of unknown causation. This is strikingly illustrated by those instances in which stoppage was not obtained, although the conditions seemed to be most favorable for its development (in this connection see p. 177).

In two experiments (N. S. V, 3, and VII, 4, 5) the longest ventricular cycle of stoppage was so protracted that, fearing the death of the animal, the ventricles were made to contract by means of mechanical or electrical irritation until they began to beat spontaneously. In one of these instances (N. S. V, 3) the ventricles failed to contract for so long a period that respiratory convulsions appeared and the auricles began to fail. At this time, fifty seconds after the onset of stoppage, the ventricles were made to beat by tapping them occasionally with a flat instrument. This mode of stimulation was continued for seventy-five seconds, when the ventricles began to beat spontaneously, at first slowly, but with gradually increasing rate, until the usual constant rate obtained.

#### CAUSE OF THE PHENOMENON OF STOPPAGE OF THE VENTRICLES.

**Theoretical considerations.** — The question now arises, What is the cause of the phenomenon of stoppage of the ventricles? In this connection two possibilities suggest themselves for consideration :

1st. The ventricles cease to beat for a time when they are no longer whipped into action by their physiological stimulus, because some time is required for the dormant inherent rhythmicity of the ventricles to acquire its maximum efficiency. In other words, the ventricles of the warm-blooded heart show a rhythm of development when they are severed physiologically from the rest of the heart

This phenomenon, long known as a consequence to ligation of the sino-auricular junction in the case of the cold-blooded heart, was first observed by Stannius<sup>1</sup> in 1852, and is described by Gaskell as follows: "In the tortoise . . . the standstill after the removal of the sinus is not permanent; the auricle and the ventricle commence after a time to beat again, at first slowly, then gradually quicker and quicker, until at last a good steady rate of rhythm is observed. . . . If the cut is anywhere between the sinus and the ventricle, the same thing may be observed; the difference being that the primary standstill is longer the nearer to the ventricle the cut is made; and the rate of the rhythm finally obtained is liable to be less the nearer to the ventricle."<sup>2</sup> "The part in connection with the sinus continues its regular beat; the part separated from it remains still for a variable time, and then, according to its inherent rhythmical power, develops a rhythmical beat of its own, the rate of that rhythm when fully developed, and the length of time that the standstill lasts, being correlated with the rhythmicity of the tissue."<sup>3</sup>

2nd. The ventricles stop beating because the clamp presses upon, or irritates in some other way, a peculiarly sensitive region stimulation of which inhibits the ventricles alone.

It is thus seen that we undoubtedly have to do here with the much debated question of the cause of the standstill of the auricle and ventricle which follows the first ligation of Stannius in the cold-blooded heart; namely, Is this standstill due to the blocking of the stimuli which arise in the venous end of the heart, or is it due to the stimulation of an inhibitory mechanism?<sup>4</sup>

**Discussion of results.** — Practically all of the experiments of this research have been planned with the object of determining which of these two possible explanations of stoppage of the ventricles is the correct one. And it might be stated here that the evidence which has been obtained is overwhelmingly in favor of the first possibility; namely, it is a manifestation of a general phenomenon which is spoken of under the term of the rhythm of development.<sup>5</sup> This evidence follows.

<sup>1</sup> STANNIUS: *Archiv für Anatomie, Physiologie und wissenschaftliche Medicin*, 1852, p. 87.

<sup>2</sup> GASKELL: Schäfer's Text-book of physiology, 1900, ii, p. 175.

<sup>3</sup> The same, p. 176.

<sup>4</sup> The literature of this subject has been recently reviewed by HOFMANN: *NAGEL'S Handbuch der Physiologie*, 1905, i, 226.

<sup>5</sup> LOHMAN (*Archiv für Physiologie*, 1904, Suppl., p. 265), working with the terrapin, refers the phenomenon of development of rhythm to the bridge fibres.

DOES STOPPAGE RESULT BECAUSE STIMULI FAIL THE  
VENTRICLES?

Stoppage of the ventricles occurs at the moment heart-block becomes complete. — In the first place attention should here again (see above) be called to the fact that when stoppage of the ventricles is obtained it is invariably synchronous with the establishment of complete block. This in itself is strong presumptive evidence in favor of the view that stoppage occurs because the normal stimuli fail the ventricles.

The relation of the duration of stoppage of the ventricles to the stage of heart-block from which it develops. — If the phenomenon of stoppage of the ventricles occurs because the mammalian ventricles require some time to develop their maximum inherent rhythm when the normal stimuli fail to reach them, we should naturally expect such stoppage to be longest when it follows immediately upon a period in which the ventricles have been beating with a rapid rate. For under such circumstances, adopting the suggestion of Gaskell, the suppression of the function of rhythmicity would be greatest, and more time would consequently be required for its full development.

It has been shown that this is actually the case (p.172; see Table I). Thus, in one and the same experiment, stoppage of the ventricles is longest when it immediately succeeds a normal 1 : 1 rhythm. It is much shorter when it follows upon a 2 : 1 rhythm. And if it has been obtained at all from a prolonged 3 : 1 or slower rhythm, such stoppage has been very short. Indeed, it appears, although this is a point difficult of demonstration, that the ventricles are, so to speak, prepared to take on their maximal inherent rhythm by an intervening period of slow (2 : 1, 3 : 1, etc.) rate. Thus, a ventricle which shows a long rhythm of development when the block suddenly becomes complete, may have a comparatively short rhythm of development when the block becomes complete through a few 2 : 1 or 3 : 1 cycles (N. S. V, 2 and 3; N. S. VII, 4 and 5).

It is possible that this phenomenon is identical with that observed by Gaskell, working with isolated strips of terrapin ventricle.<sup>1</sup> Gaskell found that such a strip would develop spontaneous contractions sooner when it was made to beat occasionally by means of electrical stimulation than when it was not so stimulated. It should here be stated that the spontaneous beats obtained by Gaskell were probably caused by applications of salt solution

<sup>1</sup> GASKELL: *Journal of physiology*, 1883, p. 43.

which he used to prevent drying of the strips. For strips of the ventricular muscle of the terrapin are not automatically contractile under normal conditions.<sup>1</sup>

But the above-mentioned relation of the duration of stoppage to the stage of block from which it develops does not suffice to demonstrate conclusively that stoppage results from the absence of stimulation. In order to prove that such is the case it is necessary to demonstrate that when the ventricles are removed from the influence of rhythmic stimuli by some method which does not in itself tend to stimulate the ventricles, they will still exhibit the phenomenon of stoppage. That this they will do we have been able to show in two ways.

1. **Stoppage from fatigue (?) of the auriculo-ventricular bundle.** — In several experiments (see p. 183) we have shown that typical stoppage of the ventricles may be obtained by increasing the rate of auricular beats at a time when conduction through the auriculo-ventricular bundle is presumably somewhat impaired. The ventricular standstill which is obtained by this method is due, we believe, to absence of stimuli. These are blocked because there exists a complete functional insufficiency of the auriculo-ventricular bundle, the result of a too rapid recurrence of impulses to be conducted through it.

(2) **Behavior of the ventricles in complete heart-block upon the cessation of rhythmic stimulation.** — To study the effect of cessation of rhythmic stimulation of the ventricles, complete heart-block is first produced and then, after the rate of the ventricles has become constant, they are made to beat more rapidly by stimulating them with rhythmic induction shocks conveyed to them through the myocardiograph. The rate of stimulation usually chosen was approximately that of the auricles of the same heart (N. S. XII, 11-13; N. S. XIV, 24, 32; N. S. XV, 27, 28; N. S. XVI, 36-41; etc.).

Upon the cessation of such stimulation the ventricles stop beating for a longer or shorter period of time, and then gradually develop the rate which they had previous to stimulation (Fig. 4). The duration of the stoppage depends largely upon the rate and duration of the preceding period of stimulation (see N. S. XVI, 36, 38, 39, and 40). In addition, the duration seems to be to some extent dependent upon the condition of the heart at the time of stimulation. Thus such stoppages have as a rule increased in length toward the close of prolonged

<sup>1</sup> HOWELL: This journal, 1902, vi, p. 181.



experiments, when the inherent rhythmical power of the ventricles, judged by their constant rate, is low<sup>1</sup> (see XV, 25-28).

It is interesting to note that the stoppages which follow rhythmic stimulation may be of either of the two types described on page 156: the longest ventricular cycle may be the first following the cessation of stimulation, or the ventricular cycles may increase in length through a few contractions. By far the most common form is one in which the longest ventricular cycle is preceded by but one ventricular cycle of not more than one or two seconds' duration. The ventricular beats then gradually become more frequent until the inherent rate of the ventricles obtains. The longest ventricular cycle of the stoppages obtained by this method lasted 15.4 seconds (N. S. XIV, 37). It followed 218 stimuli at the rate of about 150 per minute.

Facts having the same significance have been obtained by stimulating the independent ventricles with the constant current (N. S. VII, 19). As long as the current flows the ventricles beat, often with perfect rate and rhythm, but at the moment the circuit is opened they stop beating, and their rate then increases gradually just as it usually does after stoppages produced by other methods.

The type of stoppage in which the longest ventricular cycle is reached through a short series of cycles of increasing duration is rather remarkable. It indicates that although rhythmic stimulation reduces the automaticity of the ventricles

<sup>1</sup> This fact is of interest in connection with the relation of stoppage of the ventricles to the action of the vagi upon the ventricles (see below). For it was pointed out by HOUGH (*Journal of physiology*, 1895, xviii, p. 172) that more prolonged inhibition is obtained with the weak, slowly beating heart than with the strong, active heart.

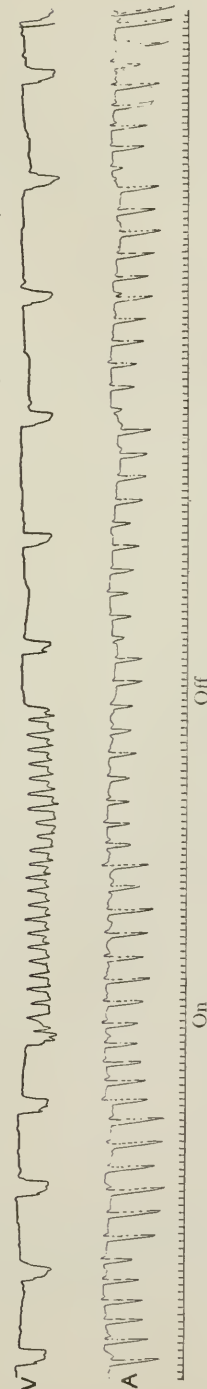


FIGURE 4.—Two-thirds the original size. Slowing of the ventricles following rhythmic stimulation of the ventricles during complete heart-block. N. S. XVI, 40.



so that they tend to cease beating upon the cessation of stimulation, this automaticity is not at its lowest level at the moment of withdrawal of stimulation, but diminishes more or less gradually thereafter. The phenomenon reminds one of that noted by Ludwig and Luchsinger,<sup>1</sup> who first showed, in the terrapin, that increased intraventricular pressure increases the rate of the isolated ventricles, but not at once, and, *vice versa*, that the rate of the ventricles does not diminish at once after lowering the intraventricular pressure.<sup>2</sup>

#### IS STOPPAGE OF THE VENTRICLES A RESULT OF STIMULATION OF AN INHIBITORY MECHANISM?

In the foregoing section it has been shown that stoppage of the ventricles may be explained satisfactorily upon the assumption that the ventricles develop their rhythm gradually when they are severed from the rest of the heart. Nevertheless, in view of the suggestion that stoppage might be an inhibitory phenomenon, the attempt has been made to determine the effect upon the ventricles, in particular, of stimulating the tissue included in the clamp. For this purpose mechanical and electrical stimulations have been employed.

**Effects of mechanical stimulation of the auriculo-ventricular bundle.**—The effect of mechanical stimulation of the tissue included in the clamp was tested as follows: In the first place it should be stated that for this purpose experiments were chosen in which the heart responded regularly and promptly to clamping, *i. e.*, in which stoppage appeared and the subsequent block disappeared promptly upon clamping and unclamping, respectively. In such an experiment complete heart-block was first produced, the clamp immediately loosened, and, before the block had disappeared, suddenly and sharply tightened. To this the ventricles, as a rule, responded with one or more extrasystoles: never was anything resembling stoppage of the ventricles obtained (XXIII and XXXI).

**Effects of electrical stimulation of the auriculo-ventricular bundle.**—For the purpose of testing the effect of electrical stimulation, the clamp was converted into stimulating electrodes. All of the hook, excepting the barb, was heavily coated with shellac. The brass block

<sup>1</sup> LUDWIG and LUCHSINGER: *Archiv für die gesammte Physiologie*, 1881, xxv, p. 232.

<sup>2</sup> CUNNINGHAM and WINTERNITZ, working in this laboratory, have shown that this phenomenon may be obtained in a striking way with the isolated ventricle of the terrapin which has been brought into just the right state of irritability by means of irrigation with 0.7 per cent sodium chloride solution followed by RINGER's solution.

of the clamp was replaced by one of hard rubber, and the edge directed toward the barb was armed with a small platinum plate. This plate was connected, through the block, with a small binding post situated on the upper edge of the block. Another binding post was attached to the free end of the brass bar which carries the hook. After the clamp had been successfully placed upon the heart, the terminals of an induction coil were connected with the two binding posts. When this clamp was tested upon the tongue, it was found that the current passed between the point of the hook and the edge of the block directed toward it only.

Tetanic stimulation of the tissue included in the grasp of this clamp never reproduced the picture of stoppage of the ventricles, neither when the sequence was normal, nor after complete heart-block had been produced. With strengths of stimulation short of those that caused fibrillation, the ventricles usually responded with frequent contractions, which were often irregular in force and rhythm. But the result obtained was rarely constant (XXIV, XXV, XXVI, XXVIII).

**Stoppage of the ventricles after atropine.** — Finally, it has been shown that the phenomenon of stoppage may be obtained after complete atropinization of the heart. For these tests, again, experiments have been chosen in which stoppage could be regularly obtained upon clamping (N. S. I and V). Atropine was then given intravenously until maximal stimulation of the vagus nerve produced no, or but an insignificant, slowing of the heart-rate. Upon clamping at such a time, stoppage was still constantly obtained. It is true that in the most successful of these experiments (N. S. I) the stoppages obtained after atropinization were not so long as those obtained before. However, it may be seen in the table that this was merely the result of a steady diminution in the duration of the stoppages as the experiment proceeded. And the fact that stoppage lasting eighty seconds has been obtained at a time when maximal stimulation of the vagus had no influence upon the heart-rate proves (N. S. V, 21), provided the accepted explanation of the action of atropine be correct, that stoppage of the ventricles is not the result of inhibition.

#### ON THE ACTION OF THE VAGI UPON THE VENTRICLES.

**Theoretical considerations.** — The statement is made by Tigerstedt<sup>1</sup> that the vagi must necessarily act upon the ventricles of the mamma-

<sup>1</sup> TIGERSTEDT: *Physiologie des Kreislaufes*, 1893, p. 250.

lian heart because, when the vagi are stimulated, the ventricles stop beating, whereas, if the ventricles be isolated physiologically from the auricles, the former will almost at once assume a constant and regular rate. This is the result which has been obtained by all investigators who have severed the connection between the auricles and ventricles by means of mass ligatures, or with the atriotome.<sup>1</sup> It is, therefore, perfectly evident that the facts at Tigerstedt's disposal justified him in his conclusion. But since it has been shown, with the aid of more satisfactory methods, that when complete heart-block is more or less suddenly established, the ventricles of the dog's heart usually stop beating for a variable, often a long, period of time, Tigerstedt's argument must necessarily fall to the ground.

Other evidence which seems to indicate that the vagi exert a chronotropic influence over the ventricles is not convincing. It is not our purpose to review the literature of this subject, for it has recently been brought up to date by Hofmann.<sup>2</sup> We wish to refer to but one phase of the subject here. Tigerstedt believes that the experiments of François-Franck, who obtained inhibition of the ventricles during fibrillation of the auricles, prove that the vagi act upon the ventricles. But, when viewed in the light of the recent experiments of Philips,<sup>3</sup> François-Franck's experiments can no longer be considered conclusive. For Philips has shown that, in all probability, some of the contractions of the ventricles during fibrillation of the auricles are caused by the passage of impulses over the auriculo-ventricular junction, and that strong stimulation of the vagi may

<sup>1</sup> WOOLDRIDGE: *Archiv für Physiologie*, 1883, p. 522; TIGERSTEDT: *Archiv für Physiologie*, 1884, 497; KREHL and ROMBERG: *Archiv für experimentelle Pathologie und Pharmakologie*, 1892, xxx, p. 49.

(Note during proof-reading.) We have recently discovered the following statements in an article by H. E. Hering entitled "Ueber die Wirksamkeit der Accelerans auf die von den Vorhöfen abgetrennten Kammern isolirten Säugethierherzen" (*Centralblatt für Physiologie*, 1903, XVII, p. 1): "Schneidet man am schlagenden Herzen die Vorhöfe weg, so stehen die Kammern nach dem letzten Schnitt trotz fortbestehender Durchströmung einige Zeit hindurch still und schlagen nach dieser Pause seltener als vor der Vorhofabtrennung. Ob der letzte Schnitt die Vorhofscheidewand oder einen anderen Theil des Vorhofes getroffen hat, ist nach meinen bisherigen Erfahrungen anscheinend gleichgültig. . . . Danach scheint der nach dem letzten Schnitt erfolgende vorübergehende Stillstand der Kammern nicht die Folge einer Hemmung, sondern die Folge des plötzlichen Ausfalles der Anregung zu sein."

<sup>2</sup> HOFMANN: *Loc. cit.*, p. 264.

<sup>3</sup> PHILIPS: *Archives internationales de physiologie*, 1905, ii, p. 271.

diminish the fibrillary contractions of the auricles. This, he found, is associated with a diminution in the rate of the ventricles whose contractions at the same time tend to become regular in rhythm.

It should be stated here that we have been able to substantiate in another way Phillips' conclusion that the irregular contractions of the ventricles during fibrillation of the auricles are caused by impulses arising in the auricles. In our experiment (N. S. XXI) conduction through the auriculo-ventricular bundle was reduced, by means of the clamp, to a stage in which the ventricles responded perfectly to auricular impulses of normal rate, but in which complete block with stoppage of the ventricles occurred when the auricular rate was increased by stimulation of the auricles with weak induction shocks. When the heart regularly exhibited this reaction, it was found that typical stoppage of the ventricles occurred whenever the auricles were thrown into fibrillary contractions.

It is therefore evident that the question of the cause of stoppage of the ventricles upon stimulation of the vagi is still an open one. The following discussion will show that much light is thrown upon this problem by a study of the response of the ventricles in heart-block to stimulation of the vagi.

**The relation between the duration of ventricular stoppage produced by clamping and by vagus stimulation.**—When the vagus nerve is stimulated tetanically with maximal stimuli, the duration of the longest ventricular cycle of the stoppage which usually results, diminishes as the degree of block increases (see Table I). Thus the duration of the longest ventricular cycle is by far the greatest when the auriculo-ventricular rhythm is 1:1; it is much less when the rhythm is 2:1; often no slowing of the ventricles is obtained when the rhythm is 3:1; and when the ventricles are beating independently of the auricles, no, or but an insignificant, slowing of the ventricles can be obtained.<sup>1</sup> The cause of this insignificant slowing which is occasionally seen, has not been determined. It may either be an effect of the diminished intraventricular tension consequent to the inhibition of the auricles, or it may represent the actual extent of chronotropic influence which the vagi exercise over the ventricles.

The results given in the foregoing paragraph may be interpreted in one of two ways:

1. It may be assumed that the vagi exert no marked chronotropic influence over the ventricles. If such is the case, the inverse relation between the duration of stoppage of the ventricles during

<sup>1</sup> There has been but one exception to this rule: it occurred in Experiment XV.

TABLE I

SHOWING THE RELATION BETWEEN STOPPAGE OF THE VENTRICLES RESULTING FROM  
OF THE

No. of experiment.	Date.	Was complete block obtained?	Through what stages of partial block.	Was stoppage obtained?	Sequence before stoppage.	Mode of recovery from stoppage.	Duration of longest Vs. of stoppage.
I.	Feb. 1	0	....	....	....	....	....
II.	7	0					
III.	9	+	(?)	0	....	....	....
IV.-VII.	"	0					
VIII.	15	+	(?)	(?)	....	....	....
IX.-XIII.	"	0					
XIV.	25	+	(?)	0	....	....	....
XV.	28	+	2 : 1 & 3 : 1	0	....	....	....
XVI.	March 2	0	....	....	....	....	....
XVII.	3	+	(?)	0	....	....	....
XVIII.	4	+	2 : 1 & 3 : 1	0	....	....	....
XIX.	6	+	2 : 1	0	....	....	....
XX.	7	+	(?)	0	....	....	....
XXI.	8	+	0	+	1 : 1	Not stated.	(?)
"	"	+	0	+	1 : 1	Permanent compl. bl.	27 + +
XXII.	17	+	0	+	1 : 1	" " "	22.8 sec.
XXIII.	24	+	Stoppage	+	Few 2 : 1	Compl. bl., 2 : 1, 1 : 1	20 sec. +
XXIV.	25	+	Long 2 : 1	0	....	....	....
XXV.	28	+	Few 2 : 1	Slight	Few 2 : 1	Compl. bl., 2 : 1, 1 : 1	....
XXVI.	30	....	....	+	1 : 1 (?)	(?)	Long
"	"	+	From stoppage	+	1 : 1	Compl. bl., 2 : 1	72.6
XXVII.	31	+	Long 2 : 1	0	....	....	....



TABLE I.

COMPRESSION OF THE AURICULO-VENTRICULAR BUNDLE AND FROM STIMULATION VAGUS NERVE.

Duration of longest Vs. during vagus stimulation.						During complete block. Reduction of beats per minute.	Remarks.
During 1:1 rhythm.	Was Vs. caused by As.	During 2:1 rhythm.	Was Vs. caused by As.	During 3:1 rhythm.	Was Vs. caused by As.		
....	....	....	....	....	....	....	Ligatures.
+	....	....	....	+	....	0	While passing ligature auricles went into fibrillary contractions, but soon recovered. No tracings made.
+	0	....	....	....	....	Incr.	1st exp. with vise-clamp. No time record.
....	....	....	....	....	....	0 to 2 +	
8 - 10 (?)	0	....	....	....	....	2 to 13 (29%)	1st exp. with fish hook clamp. Block not obtained because of fault in clamp. Animal died shortly after beginning of exp.
....	....	....	....	....	....	....	
....	....	....	....	....	....	....	
9	0	....	....	....	....	0 to 1	
31	+	....	....	....	....	1 to 4	
....	....	....	....	....	....	2.5	Complete block throughout exp.
....	....	....	....	....	....	....	No determination of max. duration of inhibition.
29 + +	+	....	....	....	....	0 to 2	Beginning of stoppage not obtained on tracing.
....	....	....	....	....	....	....	No tracings made.
....	....	....	....	....	....	....	Clamp electrode used.
....	....	....	....	....	....	....	Clamp electrode used. Atropine injected before placing clamp. No tracings made.
....	....	....	....	....	....	....	Clamp electrode used.
....	....	....	....	....	....	....	Clamp electrode used.



TABLE I

No. of experiment.	Date.	Was complete block obtained?	Through what stages of partial block.	Was stoppage obtained?	Sequence before stoppage.	Mode of recovery from stoppage.	Duration of longest Vs. of stoppage.
XXVIII.	April 4	+	0	0	....	....	....
"	"	+	0	0	....	....	....
"	"	+	0	0	....	....	....
XXIX.	5	+	Long stages	0	....	....	....
"	"	+	Short "	+	Not stated	Compl. bl.	
XXX.	12	+	From stoppage	+	1 : 1	Compl. bl., 2 : 1	29 As.
"	"	+	Long 2 : 1	0			
XXXI.	15	+	Few 2 : 1	(?)	....	....	....
"	"	....	....	+	1 : 1	Few 2 : 1	(?)
"	"	....	....	+	1 : 1	Few 2 : 1	16 sec.
"	"	....	....	+	1 : 1	Compl. bl., 2 : 1, 1 : 1	17 sec.
"	"	+	From stoppage	+	1 : 1	Compl. bl.	
NEW							
I.	June 8			+	1 : 1	Partial bl.	275
"	"	+	From stoppage	+	1 : 1	Compl. bl., 7 of 2 : 1	225
"	"	+	" "	+	1 : 1	" " 10 of 2 : 1	220
"	"	+	" "	+	1 : 1	" " (?)	150
"	"	+	" "	+	1 : 1	" " 20 of 2 : 1	105
II.	10	+	2 : 1	0	....	....	....
"	"	+	1 : 1	0			
III.	12	+	Not stated	0	....	....	....
IV.	12	+	" "	0	....	....	....
V.	13	....	....	+	16 of 2 : 1	7 of 3 : 1, 20 of 2 : 1	125
"	"	+	From stoppage	+	7 of 2 : 1	Compl. bl., long 2 : 1	250
"	"	....	....	+	1 : 1	5 of 3 : 1, long 2 : 1	53, 80, 90

(continued).

Duration of longest Vs. during vagus stimulation.						Remarks.	
During 1 : 1 rhythm.	Was Vs. caused by As.	During 2 : 1 rhythm.	Was Vs. caused by As.	During 3 : 1 rhythm.	Was Vs. caused by As.		During complete block. Reduction of beats per minute.
4 sec.	....	....	....	....	....	0 (?)	No tracings made.
0	....	....	....	....	....	....	After atropine.
0	....	....	....	....	....	....	After atropine.
....	....	....	....	....	....	....	No tracings made.
....	....	4 sec.	(?)	....	....	0 (?)	No tracings made.
9 - 20 sec.	(?)	....	....	....	....	....	No tracings made.
4.4 sec.	(?)						
4.8 sec.	(?)						
		4.2 sec.	(?)				

SERIES.

+	+						
0	+	....	....	....	....	....	After atropine.
0	+						
....	....	....	....	....	....	....	Stoppage during complete block.
....	....	....	....	....	....	....	Severe hemorrhage: experiment discontinued.
....	....	....	....	....	....	....	Exp. discontinued because stoppage was not obtained.
....	....	Not compl.	....	....	....	....	V. made to beat 375 by mechanical stimulation.
0	....	....	....	....	....	....	After atropine.

TABLE I.

No. of experiment.	Date.	Was complete block obtained?	Through what stages of partial block.	Was stoppage obtained?	Sequence before stoppage.	Mode of recovery from stoppage.	Duration of longest Vs. of stoppage.
V.	13	....	....	+	1 : 1	1 : 1	50
"	"	....	....	+	1 : 1	1 : 1	76.8
"	"	....	....	+	Alternate 2 : 1-3 : 1	1 : 1	400
VI.	14	+	Not stated	0	....	....	....
VII.	16	....	....	+	13 of 2 : 1	9 + of 2 : 1	49 +
"	"	+	Long 2 : 1 & 3 : 1	+	2 of 2 : 1	1 : 1	400 +
VIII.	17	+	x of partial bl.	0	....	....	....
IX.	17	+	Long partial bl.	0	....	....	....
X.	18	+	Not stated	0	....	....	....
XI.	20	+	From stoppage	+	Not stated	Compl. bl.	....
XII.	21	+	1 : 1	0	....	....	....
XIII.	21	+	Long 3 : 1	0	....	....	....
XIV.	23	+	Partial bl.	0	....	....	....
"	"	+	From stoppage	+	8 of 2 : 1 (?)	Long compl. bl.	17
"	"	+	(?)	0	....	....	....
"	"	....	....	+	1 : 1	Compl. bl., 3 : 1, etc.	12.8 + +
"	"	....	....	+	1 : 1	Long compl. bl., 2 : 1	56.8
XV.	24	+	26 of 2 : 1	0	....	....	....
"	"	+	6 of 2 : 1	0	....	....	....
"	"	+	From stoppage	Slight +	2 : 1 5 of 3 : 1 6 of 4 : 1	Long compl. bl.	14.5
"	"	+	Long 2 : 1 (?)	0	....	....	....
XVI.	26	+	Not stated	0	....	....	....
"	"	+	From stoppage	+	Developed gradually	Long compl. bl.	7.4
"	"	+	" "	+	Long 2 : 1	" " "	10.9

(continued).

Duration of longest Vs. during vagus stimulation.						Remarks.	
During 1:1 rhythm.	Was Vs. caused by As.	During 2:1 rhythm.	Was Vs. caused by As.	During 3:1 rhythm.	Was Vs. caused by As.		
0	....	....	....	....	....	....	After atropine and curare.
Slight	....	....	....	....	....	....	Spontaneous stoppage.
....	....	....	....	....	....	....	Exp. discontinued because stoppage was not obtained.
....	....	....	....	....	....	....	At time ventricle recovered, auricular rate very slow, — 60 per min.
Slight	0	0 (?)	0	....	....	....	No tracings made.
....	....	....	....	....	....	....	Neither vagus irritable: exp. discontinued.
....	....	....	....	....	....	....	Cat.
....	....	....	....	....	....	Slowed decidedly	Rabbit.
....	....	....	....	....	....	0	Block permanently complete.
....	....	....	....	....	....	0	Block early became complete and remained so.
47	0	7.6	0	....	....	0	
27	+	10.6	0	Slight	0	0	Block developed spontaneously and no notice was taken of the stages preceding it.
31.5	0 (?)	....	....	....	....	....	Beginning of block not recorded: it was much longer than figures indicate.
....	....	23.7	0	....	....	....	
12.2	0	....	....	....	....	0	
44	0	....	....	....	....	....	
28	+	....	....	....	....	....	
21	+	....	....	....	....	....	
....	....	....	....	....	....	0	
....	....	8.7	0	....	....	0	Block developed spontaneously.
....	....	....	....	....	....	0	Block developed spontaneously.

TABLE I

No. of experiment.	Date.	Was complete block obtained?	Through what stages of partial block.	Was stoppage obtained?	Sequence before stoppage.	Mode of recovery from stoppage.	Duration of longest Vs. of stoppage.
XVI.	26	+	From stoppage	+	Long 2 : 1	Long compl. bl.	10.7
"	"	+	20 of 2 : 1	Slight	20 of 2 : 1	" " "	7.1
XVII.	28	+	From stoppage	+	1 : 1	" " "	15.1 + +
"	"	+	" "	+	Not stated	Compl. bl.	46 + +
XVIII.	29	+	8 of 2 : 1	0	....	....	....
"	"	+	3 of 2 : 1	0	....	....	....
XIX.	30	+	From stoppage	+	1 : 1	Compl. bl., 3 + of 2 : 1	8th Vs. = 21.9
"	"	+	" "	+	1 : 1	Compl. bl., 4 of 2 : 1	5th Vs. = 11.6
"	"	+	" "	+	} See remark + of 3 : 1, 4 of 4 : 1	Compl. bl., 4 of 2 : 1	3rd Vs. = 10.2
"	"	+	" "	+		1 : 1	Compl. bl.

(continued).

Duration of longest Vs. during vagus stimulation.							Remarks.
During 1 : 1 rhythm.	Was Vs. caused by As.	During 2 : 1 rhythm.	Was Vs. caused by As.	During 3 : 1 rhythm.	Was Vs. caused by As.	During complete block. Reduction of beats per minute.	
....	....	10.7	0	....	....	0	Block developed spontaneously.
17.5	0	8.2	0				
8.6	+	....	....	....	....	....	Beginning of stoppage not recorded.
....	....	....	....	....	....	....	
{ 15.7 8.9	{ 0 +	[Alternate 10.3 8	2 : 1 & 1 : 1 0 + (?)	0	0	....	Ventricular rate in compl. bl. remarkably rapid. Thus during 1 : 1 V. rate = 4 in 14.2 and in compl. bl. 4 in 21.8.
{ 12.4 - 14.8 11.2	{ + +			0	0	....	
1st Vs. = 46	0	....	....	....	....	....	During stoppage V. rhythm is usually quite irregular, and a similar irregularity is often seen during vagus inhib. This makes it difficult to determine satisfactorily the max. duration of both.
{ 1st Vs. = 16.8 0 (?) 2nd Vs. = 7.9 0 (?)							
{ 1st Vs. = 31 0 1st Vs. = 34.4 0		....	....	....	....	....	
{ 2nd Vs. = 15.8 + 3rd Vs. = 11.8 0 (?)		4th Vs. = 6.8	0	....	....	0	Just before the block became complete, marked incr. in heart-rate for 8 cycles: then 7Vs. slightly irregular in rhythm, approx. 1 Vs. to 1½ As'.



stimulation of the vagi and the degree of heart-block, might be explained upon the assumption that the ventricles stop beating during vagus stimulation because, the auricles being inhibited, the normal stimuli fail to reach the ventricles, and these consequently stop beating until their inherent rhythm develops. (For further discussion see below.)

2. On the other hand, it may be assumed that the vagi do act forcibly upon the ventricles, but, this action being transmitted through the auriculo-ventricular bundle only, it diminishes as the functional capacity of the bundle diminishes.

We know of no method of deciding this question beyond doubt, but all of the evidence obtained by ourselves and others favors the first proposition.

*a.* There is no satisfactory evidence that the vagi can slow the ventricles of cold-blooded animals.

*b.* If the vagi act upon the ventricles, is it not permissible to presume that this action is conveyed to them through distinct nerve trunks? But no such trunks can be found in or near the auriculo-ventricular bundle.<sup>1</sup>

*c.* Furthermore, the facts already presented seem to point to a distinct tendency for the longest ventricular cycles obtained during complete vagus inhibition of the auricles and in stoppage of the ventricles to be of the same duration in each of the various stages of heart-block. It is a difficult matter to obtain exact data bearing upon this relation, for rarely can exactly comparable conditions be obtained in one and the same experiment. Thus, when, during vagus stimulation, the first ventricular cycle is the longest, it usually exceeds the longest ventricular cycle of that type of inhibition in which the longest cycle is reached through 1, 2, or 3 cycles of increasing duration. And the same variations are seen in connection with the phenomenon of stoppage of the ventricles. Furthermore, one cannot always be certain that inhibition of the auricles is complete, *i. e.*, that the normal stimulus does not occasionally reach the ventricles and cause them to beat before they otherwise might have. For often evidences of auricular contractions could not be obtained from a study of the tracings, when these were detected by direct inspection of the auricles. But it should be stated that this difficulty was largely overcome by improvements in the recording apparatus as the research progressed.

<sup>1</sup> RETZER, in ERLANGER: *Journal of experimental medicine*, to appear.

But despite all of these disturbing factors, there is, as may be seen upon examination of the table (No. 1), a distinct tendency for the duration of the longest ventricular cycle of vagus stimulation to equal in duration the longest ventricular cycle of stoppage in each stage of heart-block.

In this connection it is interesting to note that strips of the ventricle of the terrapin remain quiescent indefinitely, unless they are treated with certain saline solutions.<sup>1</sup> And it is a well-established fact that the ventricles of the intact terrapin heart can be kept quiescent indefinitely by stimulation of the vagus nerve.<sup>2</sup>

Still, it might be maintained in relation to this approximate equality of stoppage of the ventricles upon clamping and during vagus stimulation, that a possible conduction of inhibition through the auriculo-ventricular bundle and the conduction of the auricular impulses through the same bundle are always affected in like degree by compression of the conducting tissue. In answer to this, it may be said that it is hardly likely that stoppage of the ventricles upon clamping, which, it has been proved, is not an inhibition, should have almost exactly the same duration as stoppage caused by a process presumedly different. The simpler explanation is that the stoppage of the ventricles during stimulation of the vagus nerve depends, as does the other form of stoppage, upon the fact that the impulses which normally cause the ventricles to beat, fail to reach them.

**The influence of the vagi upon the response of the ventricles to rhythmic stimulation.**— The fact that the duration of ventricular stoppage, induced by stimulation of the vagus, diminishes as the degree of block increases, might be accounted for in still another way. It might, for instance, be assumed that the vagi exert their influence upon the ventricles through paths other than those contained in the auriculo-ventricular bundle. When the ventricles are beating slowly, as they do in complete heart-block, anabolism (to use the term of Gaskell's theory)<sup>3</sup> is at its height, and consequently the ventricles cannot be inhibited. The following experiment was performed to test this assumption: Complete heart-block is first produced, and then the ventricles are made to beat, by means of artificial stimuli,

<sup>1</sup> HOWELL: *Loc. cit.*

<sup>2</sup> MILLS, WESLEY: *Journal of physiology*, 1885, vi, p. 259; HOUGH: *Journal of physiology*, 1895, xviii, p. 173.

<sup>3</sup> GASKELL: SCHÄFER'S *Text-book of physiology*, ii, p. 220.

with a rate approximately equal to that of the auricles of the same heart. For this purpose a strength of the rhythmic induction shocks was chosen which was so feeble that the ventricles failed to respond to some of the stimuli; in other words, the stimuli were almost minimal. While the ventricles were being stimulated in this way, maximal stimulation of the vagus had no apparent influence upon the response of the ventricles to the induction shocks (*e. g.*, XVI, 32-38). It is, therefore, evident that the failure of the ventricles to respond to stimulation of the vagus during complete heart-block does not find its explanation in the fact that the ventricles are beating slowly.

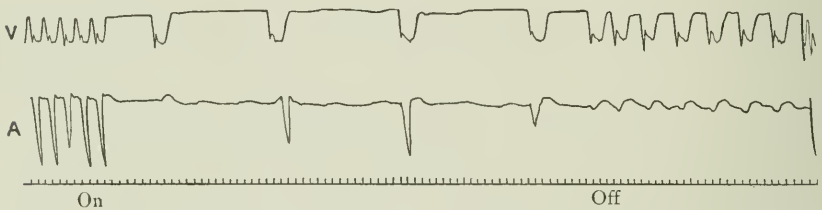


FIGURE 5.—Two-thirds the original size. Vagus stimulation: ventricular stoppage of the second type. N. S. XVI, 17.

The similarities between the types of stoppage of the ventricles produced in the several ways.—There is still another fact which indicates that the stoppages of the ventricles caused by the two above-mentioned processes are identical. It will be remembered that there are two types of stoppage. In one, the first ventricular cycle is the longest, whereas in the other the ventricular cycles gradually increase in length to a maximum. It is interesting to note that these two types occur in stoppages produced by clamping, by stimulation of the vagus and after the cessation of rhythmic stimulation of the ventricles in complete block. This in itself would be of but little significance, were it not for the fact that the same type of stoppage may be obtained more or less constantly in the same experiment. For example, in N. S. XVI, the stoppages produced in all ways were almost invariably of the more uncommon type, namely, that in which the ventricular cycles gradually reach their maximum duration (see Figs. 2, 4, and 5).

Furthermore, in one experiment (N. S. XIX) the ventricular rhythm during stoppage induced by clamping was usually quite irregular; and here the same irregularities were sometimes seen in the stoppage of vagus stimulation and in the stoppage following

rhythmic stimulation of the ventricles in complete block (see Figs. 6, 7, and 8).

It should be stated that in experiment N. S. XVI, as well as in others, the first ventricular cycle during vagus stimulation is usually longer than the first ventricular cycle of stoppage produced by clamping. This may indicate that the vagi do exert some slight chronotropic influence over the ventricles. But, on the other hand, it may be due to the fact that during vagus stimulation the inhibition of the auricles to some extent protects the ventricles from the marked increase in intraventricular tension which obtains in the other methods of producing stoppage. In addition, the ventricles are less apt to be irritated mechanically by the clamp in the case of stoppage induced through the vagus than in the other forms of stoppage.

**Summary.** — The experiments herein presented demonstrate that a temporary stoppage of the ventricles of the dog's heart may be induced by four apparently different procedures; namely, (a) by blocking mechanically the passage of impulses through the auriculo-ventricular bundle; (b) by stimulation of the vagus nerve; (c) by sudden cessation of rhythmic stimulation of the ventricles in complete block; and (d) by increasing the auricular rate during partial block. From the data which have been obtained it is evident that the

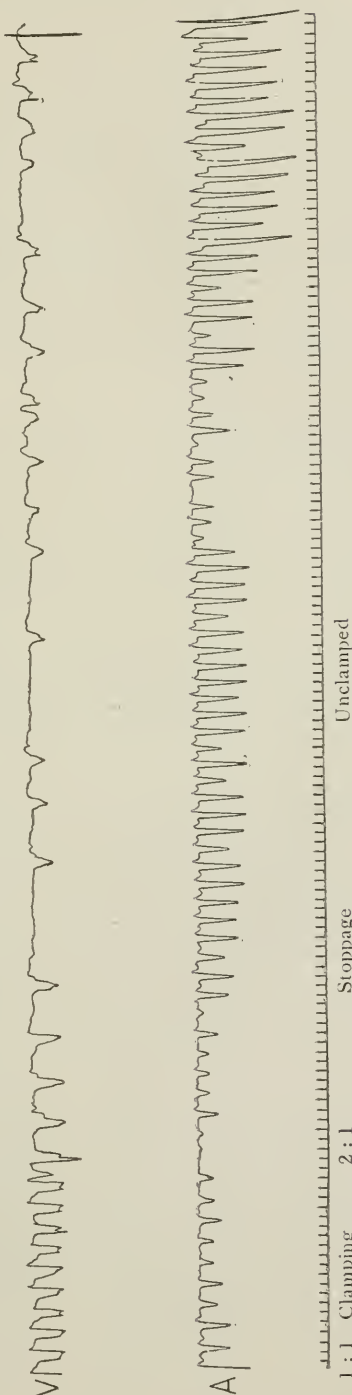


FIGURE 6. — Very slightly reduced. Ventricular arrhythmia during stoppage produced by clamping. N. S. XIX, 18.

stoppages induced in these diverse ways are due to one and the same cause, namely, the more or less sudden withholdment from the ventricles of the impulses which have been setting their pace. Under such circumstances there seems to be a gradual development of the inherent but dormant rhythm of the ventricles, so that they soon begin to beat, slowly at first, but with a rhythm which gradually increases to a constant but comparatively slow rate.

The experiments do not preclude the possibility of some slight chronotropic influence of the vagi over the ventricles, but it is cer-

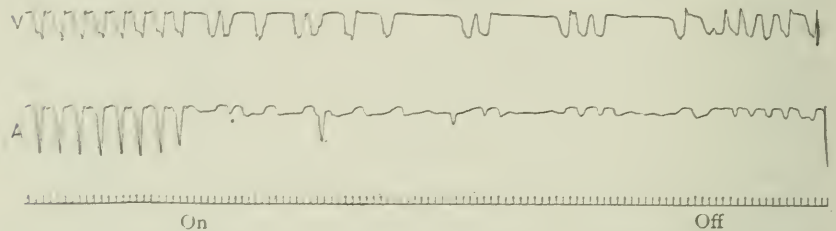


FIGURE 7. — About three-fifths the original size. Ventricular arrhythmia during stoppage produced by vagus stimulation. N. S. XIX, 17.

tain that if the vagi have such action, it is insignificant in comparison with the more striking phenomenon of development of rhythm which is associated with inhibition (?) of the auricles.<sup>1</sup>

## II. THE INFLUENCE OF VARIATIONS OF THE AURICULAR RATE UPON THE DEGREE OF HEART-BLOCK.

**Introduction.** — It was found in the instance of heart-block in man which was studied by one of the authors, that both an increase in the degree of block and the onset of stoppage of the ventricles were invariably preceded by a more or less sudden increase of the auricular rate; and it was assumed upon the basis of the experiments of Gas-

<sup>1</sup> In this connection reference should be made to one of the general conclusions reached by HERING in connection with his researches on heart-block in mammals, namely, that the vagus slows the independent ventricles. A careful perusal of his paper (*Archiv für die gesammte Physiologie*, 1905, cviii, p. 281) has failed to reveal specific references to such a result except in connection with one experiment performed upon a rabbit (experiment of March 26). (See our experiment, N. S. XI.) Nor do the curves obtained from any other of the animals experimented upon show a decided ventricular slowing upon stimulation of the vagus during complete heart-block.



kell and others, working with the cold-blooded heart, that such increases in the degree of block were indirectly due to the acceleration of the auricles. For the increase in the auricular rate is associated with an increase in the number of impulses to be conducted through the auriculo-ventricular bundle, and this, in turn, would be associated with a diminution in the functional capacity of the bundle.

**Variations in the auriculo-ventricular rhythm, caused by acceleration of the auricles.**— That the above-mentioned explanation of the variations of the degree of heart-block in man is probably correct, is indicated by analogies which have been obtained from a study of the reaction of the dog's ventricles to variations of the auricular rate in various stages of heart-block. Thus, if the conductivity of the auriculo-ventricular bundle be but slightly disturbed by moderate compression, a very slight increase of the auricular rate may convert a 1 : 1 rhythm into a 2 : 1 rhythm ; or a 2 : 1 into a 3 : 1 ; and a 3 : 1, or even a 2 : 1 rhythm, into complete block (N. S. VII, 17, and N. S. XIV, 12).

Some or all of these changes have been brought about by the following methods of increasing the auricular rate : (1) Rhythmic stimulation of the auricles with induction shocks (*e. g.*, N. S. VII, 8, 10, 12-16, Fig. 9) ; (2) Stimulation of the accelerator nerve (*e. g.*, N. S. XIV. 5) ; and (3) Pouring warm salt solution over the auricles (*e. g.*, N. S. I, 14*a*). Conversely, a diminution in the degree of block has

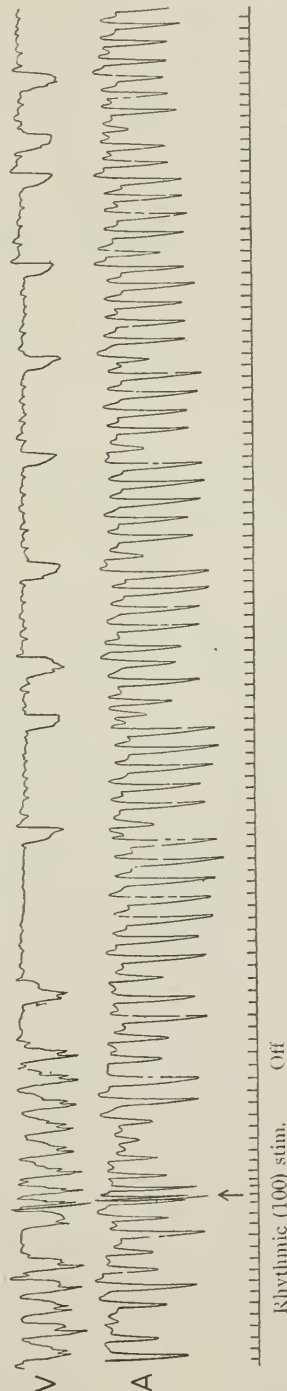


FIGURE 8.— The original size. Ventricular arrhythmia during stoppage following rhythmic stimulation of the ventricles in complete block. N. S. XIX, 26.



been obtained by slowing the rate of the auricles (1) through the vagus nerve (best seen during recovery from complete inhibition, *e. g.*, N. S. V, 4 *b*), and (2) by pouring cold salt solution over the auricular end of the heart (*e. g.*, N. S. V, 26). Usually all such changes in rhythm are temporary; the original rhythm returns after the acceleration or retardation of the auricles has passed away.

The variations in the rate of conduction through the auriculo-ventricular bundle associated with changes in rhythm; the intersystolic (As.-Vs.)

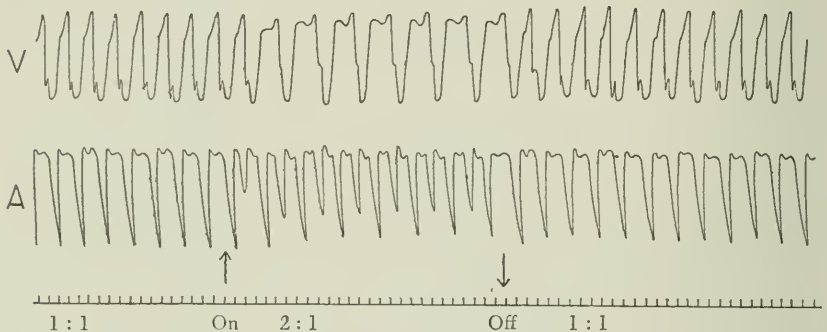


FIGURE 9.— The original size. Conversion of a 1:1 into a 2:1 rhythm by means of rhythmic stimulation of the auricles. N. S. V, 10.

period. — It is of course possible that the improvement in the conductivity of the auriculo-ventricular bundle which is seen during vagus inhibition and during recovery from vagus inhibition, is due to a direct, positive dromotropic influence of the vagus over the heart-muscle. But the results which we have obtained do not indicate that such is necessarily the case. The changes in the auricular rate in themselves apparently suffice to account for the alterations in conductivity. Comparison of Tables II, III, and IV will serve to justify this standpoint. The typical changes in heart-rate and in conductivity that occur as the result of stimulation of the vagus may be seen in Table II. The similarities between these and the results obtained by pouring over the auricles salt solution at the room temperature, illustrated in Table III, and by releasing pressure on the auriculo-ventricular bundle (Table IV), are obvious. These tables represent the results obtained in practically consecutive procedures in the same experiment.

It may be seen in each of these tables that the alterations in rhythm are preceded by alterations in the As.-Vs. intervals. These intervals increase until the block, as indicated by the auriculo-

ventricular rhythm, becomes more complete, when they suddenly diminish; and *vice versa*, the intervals diminish as the degree of block diminishes. And, in a general way, in the same rhythm the As.-Vs. intervals vary inversely as the duration of the ventricular

TABLE II.

Showing the effect on the auriculo-ventricular sequence of stimulating the vagus nerve. Maximum tetanizing current after atropine. N. S. V, 24.

No. of Vs.	A.-V. Rhythm.	Duration of V. cycles in one-fifth sec.	Duration of As.-Vs. in seconds.	Remarks.	
1	2:1	6.3	.15	Vagus stimulation begun.	
2	2:1	6.4	.13		
3	2:1	6.4	.13		
	Extra-syst.				
4	1:1	4.3	.14		
5	1:1	3.6	.13		
6	1:1	3.4	.13		
7	1:1	3.3	.13		
8	1:1	3.5+	.11		
9	1:1	3.5	.12		
10	1:1	3.4	.11		
11	1:1	3.6	.13		Vagus stimulation stopped.
12	1:1	3.5	.13		
13	2:1	6.8	.11		
14	2:1	6.7	.11		
15	2:1	6.9	.13		
16	2:1	6.7	.13		

cycles. It is impossible to recognize in these, or in any of many similar experiments, a specific dromotropic action exercised by the vagus nerve or, we might add, by the accelerator nerve.

**Stoppage of the ventricles induced by increasing the auricular rate during partial block.** — Attempts to bring on complete block, preceded by stoppage of the ventricles, by increasing the rate of the

TABLE III.

Showing the effect on the auriculo-ventricular sequence of cooling the auricular end of the heart while the rhythm is occasionally 2:1. N. S. V, 26.

No. of Vs.	A.-V. Rhythm.	Duration of A. cycles in one-fifth sec.	Duration of As.-Vs. in seconds.	Remarks.
1	2:1	—	.19	
2	1:1	3.3	.18	Pouring cold salt solution over auricles.
3	1:1	3.4	.18	
4	1:1	3.3	.2	
5	1:1	3.5	.2	
6	1:1	3.5	.2	
7	1:1	3.6	.2	
8	1:1	3.7	.2	
9	1:1	3.6	.2	
10	2:1	2.7 + 3.5	.17-	
11	1:1	3.9	.17-	
12	1:1	4.2	.17	
13	1:1	3.9	.16	
14	1:1	3.8	.18	
15	1:1	3.8	.17	
16	1:1	3.8	.16	
17	1:1	3.7	.18	Ceased pouring followed by ventricular extra-systole.
18	1:1	3.7	.18	
19	1:1	3.6	.18	
20	1:1	3.6	.18	
21	1:1	3.5	.2	
22	1:1	3.4	.2	
23	1:1	3.4	.2	
24	1:1	3.4	.2	
25	1:1	3.2	.2	
26	1:1	3.2	.2	
27	2:1	3.3 + 3.4	.17	
28	2:1	3.3 + 3.3	.17	
29	1:1	3.3	.18	
30	1:1	3.2	.2	
31	2:1	3.3 + 3.2	.17	
32	2:1	3.2 + 3.2	.17	
33	1:1	3.2	.17	

TABLE IV.

Showing the effect on the auriculo-ventricular sequence of clamping and unclamping the auriculo-ventricular bundle. N. S. V, 19.

No. of Vs.	A.-V. Rhythm.	Duration of V. cycles in one-fifth sec.	Duration of As.-Vs. in seconds.	Remarks.
1	1:1	3.3	.22	Clamping.
2	1:1	3.2	.2	
3	1:1	3.1	.2	
4	?		.25	Stoppage of V. for 57. Stoppage continued or 4:1
5	4:1	13.0	.23	
6	2:1	6.4	.25	Unclamped.
7	2:1	6.5	.23	
8	2:1	6.4	.22	
9	2:1	6.3	.22	
10	1:1	3.2	.25	
11	2:1	6.3	.22	
12	1:1	3.2	.24	
13	1:1	2.9	.27	
14	2:1	6.3	.22	
15	1:1	3.0	.24	
16	2:1	6.2	.23	
17	2:1	6.2	.25	
18	2:1	6.1	.22	
19	2:1	6.2	.2	
20	1:1	3.0	.26	
21	2:1	6.2	.2	
22	2:1	6.1	.2	
23	1:1	3.—	.23	
24	1:1	3.+	.22	
25	1:1	3.0	.25	
26	1:1	3.0	.26(?)	
27	1:1	2.9	.27	
28	2:1	6.1	.2	
29	2:1	6.1	.22	
30	1:1	3.0	.23	
31	1:1	3.1	.23	
32	1:1	3.0	.24	
33	2:1	6.1	.2	
34	2:1	6.1	.2	
35	1:1	3.1	.22	
36	1:1	3.1	.2	

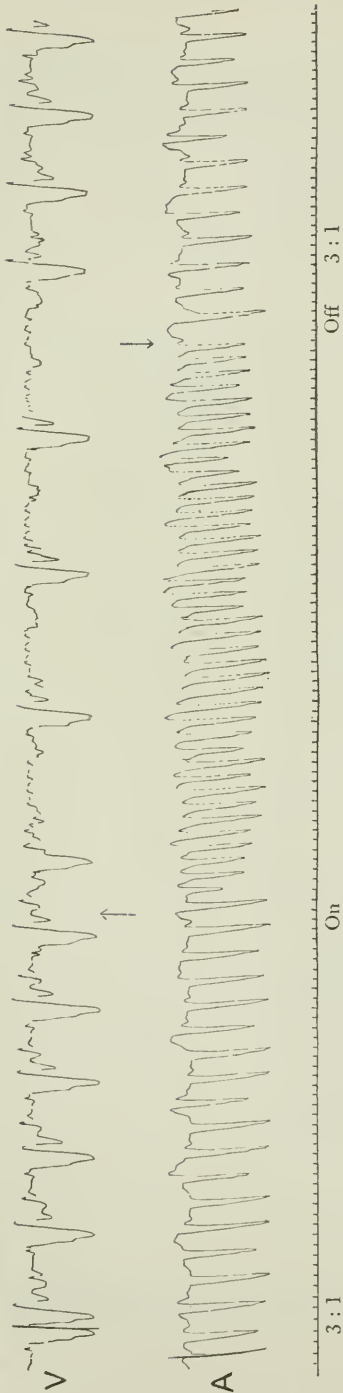


FIGURE 10. — Very slightly reduced. Slowing of the ventricles (stoppage?) associated with rhythmic stimulation of the auricles. N. S. VII, 16.

auricles at a time when there exists a high degree of partial block, have but rarely been certainly successful. In these attempts rapid, rhythmic, unipolar stimulation of the auricular appendix was employed. The results obtained may be illustrated by the following example (N. S. VII, 16; see Fig. 10). Before stimulation of the auricles, whose rate was 120 per minute, the rhythm was 3 : 1, and each ventricular cycle lasted approximately 1.5 seconds. During rhythmic stimulation of the auricles with approximately 214 stimuli per minute, the ventricles apparently responded to the ninth and nineteenth auricular contractions. At this time the longest ventricular cycle equalled 2.8 seconds; *i. e.*, the length of the cycle had been almost doubled. But here the auricular cycles are so short that it becomes difficult to determine positively whether or not the ventricular contractions are caused by auricular stimuli. Nevertheless, in some of the attempts (*e. g.*, N. S. VII, 17) the block almost certainly became complete.

This experiment (N. S. VII) was not well adapted to testing the possibility of bringing on stoppage of the ventricles from partial block by increasing the rate of the auricles, for it was one of those rare experiments in which high degrees of partial block (5 : 1 and 6 : 1 rhythms) were observed. Quite recently two experiments have been performed which clearly

prove that stoppage may be so obtained. In these experiments no tracings were made, but even without them the results were unmistakable. Thus, in one (N. S. XX) it was found that while the rhythm was 2:1, with the auricles beating at the rate of 32 per 10 seconds, rhythmic stimulation of the auricles at the rate of 42 per 10 seconds resulted in typical stoppage of the ventricles lasting 19 auricular contractions, *i. e.*, somewhat less than 5 seconds. Sometimes during rhythmic stimulation the rhythm became 3:1. But if, under such circumstances, the pressure on the auriculo-ventricular bundle was increased, but not by enough to alter the 2:1 rhythm, then rhythmic stimulation of the auricles would again cause stoppage of the ventricles.

In the other one of these experiments (N. S. XXI) the results were even more striking. Thus, while the rhythm was 1:1 with a heart-rate of about 20 beats per 10 seconds, stoppage of the ventricles lasting over 10 seconds was repeatedly obtained upon increasing the auricular rate to 38 beats per 10 seconds. At one time during the course of this experiment spontaneous alterations in rhythm occurred repeatedly in the following order: Complete block, a few cycles of 2:1, one or two cycles of 1:1, and then typical short stoppage, followed by complete block, etc. Here the stoppage was undoubtedly the result of a diminution in the conductivity of the auriculo-ventricular bundle brought on by a too frequent recurrence of normal stimuli.

**Can stoppage of the ventricles be obtained by increasing the auricular rate in complete heart-block?**—All attempts to bring on stoppage of the ventricles by increasing the rate of the auricles during complete heart-block have failed. At such times rhythmic stimulation has had absolutely no effect upon the rate of the ventricles. However, this result does not necessarily indicate that stoppage of the ventricles cannot be so obtained. For it is possible that in the experiments thus far made, the block may have been absolute: the marked slowing of the ventricles which has been obtained by increasing the auricular rate during partial block, indicates that possibly typical stoppage may be obtained under circumstances more favorable for its development.

It is perfectly obvious that the form of complete heart-block which is determined by a permanent injury of the auriculo-ventricular bundle will differ in many respects from that form of complete heart-block which is due to a functional insufficiency of the auriculo-ventricular bundle, caused, in part at least, by a relatively too rapid recurrence of impulses. We would therefore suggest



for the former the term absolute heart-block and, for the latter, relative heart-block. In neither form would auricular impulses of normal rate determine ventricular contractions. But in the relative form changes in the auricular rate might affect the rate of the ventricles. For example, a marked slowing of the auricles might cause the block to become partial, or an acceleration of the auricles might cause stoppage of the ventricles (see below); whereas in absolute heart-block changes in the auricular rate could not possibly influence the ventricular rate except, perhaps, by altering the intraventricular tension.

Theoretically, the same distinction might be made in the case of partial heart-block, but here, for obvious reasons, it would be difficult to draw a hard and fast line between them.

*Spontaneous stoppage of the ventricles during complete heart-block.* — In two experiments (N. S. II and XIX, 18 and 19), while the heart-block was complete, the ventricular rate has, for some unknown reason, slowed down much as it does in the phenomenon of stoppage. In these experiments the ventricular rhythm during complete block was never perfect. To what these irregularities were due has not been determined. But the condition has been mentioned here because the fact that the rate of the ventricles in complete block may decrease more or less abruptly, is of interest in connection with the fact that this, rather than the sudden establishment of complete block, was found to be the more common cause of the syncopal attacks in the case of Stokes-Adams disease<sup>1</sup> studied by one of the

<sup>1</sup> (Note during proof-reading.) The following observations seem to indicate that the syncopal attacks of Stokes-Adams disease result from some action of the auricles on the ventricles (see ERLANGER: *Journal of experimental medicine*, 1905, vii, p. 676):

1. JAMES MACKENZIE informs us that in a case of Stokes-Adams disease which he is following the syncopal attacks were frequent while the heart-block was partial, but since the block has become permanently complete, the syncopal attacks have ceased.

2. It has been stated that in our first case of Stokes-Adams disease the syncopal attacks, as a rule, occurred while the block was complete. Nevertheless, the observation was made (*loc. cit.*, p. 718) that these attacks were most frequent and most severe on a day when evidence had been obtained of a marked improvement in the conductivity of the auriculo-ventricular bundle. At the time no positive conclusion was reached with regard to the cause of this remarkable association. It might now be stated that although, on this day, too, ventricular stoppage occurred while the heart-block was complete, the above-mentioned fact indicates that the complete block was of the relative form.

3. We have observed in connection with our second case of Stokes-Adams disease that the syncopal attacks are becoming more infrequent as the disease progresses.

authors. In this case the ventricular stoppage was always preceded by an acceleration of the auricles. The only cause of such stoppages of which it is possible to conceive is one which was suggested in a previous paper. If it be assumed that the rate of the ventricles in relative complete block is determined by that region which possesses the highest degree of rhythmicity, then anything (possibly an increase in the number of impulses arriving in the auriculo-ventricular bundle) which could cause such a region to become functionally inactive might cause a still lower region, which would probably possess a still lower degree of rhythmicity, to set the pace for the ventricles. It is conceivable that such a shifting might be associated with all of the phenomena, including stoppage, observed when the ventricles are suddenly cut off from their normal pace-maker.

#### THE PATH TAKEN BY OTHER THAN NORMAL CARDIAC IMPULSES.

It might not be superfluous to state that our experiments indicate that impulses generated by mechanical and electrical stimulation take the same path as the normal cardiac impulses. For stimuli which caused extra contractions of either the auricles or the ventricles never crossed the auriculo-ventricular junction during complete heart-block. But during partial heart-block they frequently, although not invariably, crossed this junction, and when the conductivity was normal it was the rule for them to cross.

#### PROTOCOLS OF EXPERIMENTS.

The protocols of experiments have been abbreviated as much as was thought to be consistent with clearness. A. = auricles; V. = ventricles; As. and Vs. = A. and V. systole or cycle, according to the sense implied; As.-Vs. = intersystolic period. The unmodified numbers express the time in fifths of seconds. Autopsy notes have been omitted: the general findings are given in the body of the paper. Likewise tables have been omitted.

*Experiment 1.*—June 8, 1905. Small fox terrier. Hook passed, clamp tightened, no block. Hook reinserted in septum, clamp tightened, complete block. Unclamped: 2 : 1 lasting for some time. Clamped again: complete block with long stoppage of ventricles. Now A. and V. contractions recorded with tambours.

1. Clamping and unclamping. Rhythm 1 : 1, rate 119.8 per minute. Clamped : stoppage without preceding partial block ; 1st Vs. = 7.5 ; 2nd = 275. Rhythm during recovery not determinable ; tracings not clear.

2. Same. Rhythm 1 : 1, rate 121.3 per minute. Clamped : stoppage without preceding partial block ; 1st Vs. (longest) = 225 : A. rate toward end, 80.5 ; complete block about 1 minute with V. rate 31.3 and A. rate 110 per minute. Normal sequence returned through 7 of 2 : 1.

3. Same. Rhythm 1 : 1, rate = 120.5 per minute. Clamped : stoppage without preceding partial block ; longest Vs., 1st = 220. Normal sequence recovered through complete block and 10 of 2 : 1.

After this, stimulation of vagus gave marked inhibition. 1 mg. atropine intravenously. Now no inhibition upon stimulation of vagus. (These tests were made without tracings.)

4. Clamping and unclamping after atropine. Rhythm 1 : 1, rate = 106.2 per minute. Clamped : stoppage without preceding partial block ; longest Vs., 1st = about 150. Followed by long complete block ; record not carried further.

5. Same. Rhythm 1 : 1, rate = 109.9 per minute. Clamped : stoppage without preceding partial block ; longest Vs., 1st = 105. Normal sequence recovered through complete block and 15 of 2 : 1. 1 : 1 rhythm induced by pouring cold salt solution over heart.

6. Pouring cold salt solution over heart. Rhythm 1 : 1, rate = 113.1 per minute. Poured cold salt solution over heart : rate = 96.7 per minute.

7. Record of effect of clamping on rapidly revolving drum : nothing.

8-16. Procedures not related to present problem.

*Experiment 2.* — June 10, 1905. Small fox terrier. Hook passed and clamp tightened three times without obtaining block. Upon fourth trial obtained 2 : 1 rhythm followed by complete block without stoppage. Later 1 : 1 rhythm returned. While manipulating clamp, complete block came on at once without stoppage. During this complete block, which persisted throughout rest of experiment, ventricular rhythm slightly irregular and at one place shows definite stoppage, the duration of successive Vs<sup>1</sup>. being : 11, 20, 14.2, 18, 47 (?), 58, 13.4, 13, 18.4, 14.4, 23.2, 16.2, 23.8, 14.8, 15.6, 16.4, 14, 12.5, 11.6.

*Experiment 3.* — June 12, 1905. Small fox terrier. Clamp adjusted about ten times ; finally, on last attempt obtained block but without stoppage. But by this time animal had lost so much blood that experiment was discontinued.

*Experiment 4.* — June 12, 1905. Small fox terrier. Hook passed several times. Twice got block but without stoppage. Therefore experiment discontinued.

*Experiment 5.*—June 13, 1905. Small mongrel. Hook inserted successfully upon first attempt (see below). Myocardiograph used for recording movements of right V.

1. Clamping and unclamping: 1 : 1, 3 of 2 : 1, 12 of 4 : 1, 5 of 2 : 1, 1 : 1.

2. Clamping, etc. Rhythm 1 : 1. 16 of 2 : 1, 2 of 4 : 1, with rapidly lengthening As.-Vs., or complete block, then stoppage: 1st Vs. = 125, 2nd = 27.5; V. rate then gradually increases through 4 beats, when rhythm becomes 3 : 1 for 7 Vs'; then 20 of 2 : 1.

3. Clamping, etc. Rhythm 1 : 1; A. rate = 113.4 per minute. 7 of 2 : 1, the last two with very rapidly lengthening As.-Vs., or complete block; stoppage: 1st Vs. = 250. Toward end of this period A. rate very slow and contractions feeble; therefore ventricles were made to beat by striking them with the flat of forceps for 375 when V. began to beat spontaneously, at first in complete block with approximately 4 As'. to 1 Vs. for 3 Vs'. and then during remainder of tracing, 1 Vs. for 2½ As', approximately; V. rate = 45.6 per minute.

4, 4 a, and 4 b. Vagus stimulation. Rhythm 2 : 1 persisting from procedure No. 3. 4 b. A. rate markedly slowed (from 1 As. in 2.2 to 1 As. in 6.3), rhythm becoming 1 : 1. Every Vs. caused by an As. After: A. rate increases, V. following at 1 : 1, but soon rhythm becomes 2 : 1.

5. Rhythm becoming 1 : 1.

6-8. Vagus stimulation after administration of atropine, 1 mg., intravenously, secondary coil at 0. In No. 6 slowing for one beat; in others no effect upon heart-rate.

9. Clamping after atropine. From 1 : 1 (A. rate = 98.4 per minute) to stoppage: 1st Vs. = 8, 2nd = 53, 3rd = 80, 4th = 90; then V. rate gradually increases through 9 Vs'; then 5 of 3 : 1, and finally 2 : 1 until beginning of next number.

10. Vagus stimulation, coil at 0. Rhythm 1 : 1. A. rate slowed ¼ of a beat in 8.

(1 mg. atropine intravenously).

11. Same. Before stimulation, rate = 105.8 per minute. During stimulation, rate for 6 beats = 93.7 per minute. No measurable change in As.-Vs.

12. Same. Results similar.

13. Vagus stimulation. Rhythm 2 : 1. During stimulation rhythm becomes 1 : 1, returning to 1 : 1 after stimulation. Table omitted. Result similar to No. 14.

14. Vagus stimulation. Rhythm 1 : 1 with occasional 2 : 1 cycles (see Table II).

(Curare intravenously).

15. Vagus stimulation. Rhythm 1 : 1. No obvious effect.

16. Clamping, etc. Rhythm 1:1. Complete stoppage of V. for 50 without intermediate stages, then 1:1 without intermediate stages.

17. Same. Rhythm 1:1. Rate = 87.2 per minute. Complete stoppage of V. without intermediate stages: 1st Vs. = 32.2, 2nd = 38.6; then 1 of 2:1, followed by 1:1.

18. Same. Rhythm 1:1. Rate = 85.8 per minute. Complete stoppage without intermediate stages: 1st Vs. = 21, 2nd = 76.8. Then 1:1 without intermediate stages.

19. Clamping and unclamping (see Table IV).

20. Vagus stimulation. Rhythm 2:1. Before: A. rate = 90.9 per minute. During: rhythm still 2:1, and A. rate = 91.2 per minute.

21. Spontaneous stoppage. X of 2:1, 1 of 3:1, 3 of 2:1, 1 of 3:1, 2 of 2:1, 2 of 3:1, 1 of 6:1 (?) then V. stoppage for 400. Rhythm became 1:1 at once upon striking ventricle with flat of forceps. But at this time A. was so slow (42 per minute) that V. rate was consequently slow. As A. rate increased, rhythm became 2:1.

22. Vagus stimulation. Rhythm 1:1. Very slight slowing of heart.

23. Stoppage of the ventricles during vagus stimulation; probably accidental. Rhythm 1:1. Stoppage at once: 1st Vs. = 31.2, 2nd = 31.8. End of tracing.

24. Vagus stimulation. Rhythm 2:1. During stimulation rhythm becomes 1:1, returning to 2:1 after stimulation. Table showing variations of As.-Vs. omitted. Results similar to those in Table II.

25. Same, with similar results.

28. Effect of pouring cold salt solution over heart. Results given in Table III.

27 and 28. Effect of pouring warm salt solution over heart. Rhythm 2:1. Rate accelerated, but A.-V. rhythm unchanged.

*Experiment 6.* — June 14, 1905. Small mongrel. After passing hook several times, finally obtained complete heart-block (without stoppage) from which the heart did not recover. Therefore experiment discontinued.

*Experiment 7.* — June 16, 1905. Small mongrel. Upon third attempt block, with stoppage, obtained.

1, 2, and 3. Accelerator stimulation. Rhythm 1:1. No acceleration.

4. Clamping and unclamping. Rhythm 1:1. Rate = 99 per minute. 13 of 2:1, then stoppage. After this had lasted 49, ventricles were stimulated with induction shocks a trifle faster than A. rate. The ventricles responded to all of 7 such stimulations. At cessation of stimulation 9 of 2:1, then 1:1.

5. Same. 2 of 2:1 (?), then stoppage of V. lasting about 400 in all. During this stoppage V. was stimulated rhythmically at various times. Ventricle began to beat spontaneously about 15 after last period of stimulation. Immediate 1:1 rhythm, but A. rate now very slow, about 1 beat per second.



6. Rhythmic unipolar stimulation of right auricle. Rhythm 1 : 1. Rate of stimulation a trifle faster than normal A. rate. Rhythm remains 1 : 1; A. occasionally fails to respond to stimulation.

7. Same. Results the same.

8. Same. Rhythm 2 : 1. During stimulation rhythm changes to 3 : 1, then to 4 : 1.

9. Same. Rate of stimulation somewhat more rapid. Rhythm 1 : 1. A. and V. follow stimuli accurately.

10. Same. Rate of stimulation 12 to 8.8 As. Rhythm 1 : 1, but just after recovery from complete block. A. responds to every stimulus; V. to every other As. (It is interesting to note that although, during rhythmic stimulation, A. beats faster than normal, V. beats slower than normal.

11. Same. A. and V. follow stimuli accurately.

12. Same. Rhythm 1 : 1, just after recovery from complete block. During stimulation rhythm 2 : 1.

13. Same. Before stimulation rhythm 2 : 1 and 3 : 1. During stimulation V. rate markedly slowed, but Vs'. apparently (?) caused by As'. : 1st Vs. in 7 As', 2nd in 10 As', 3rd in 10 As'. End of stimulation: V. rate then increases thus: 1 Vs. in 5, 6, 5, and 5 As'. Longest Vs. = 18; before stimulation, during 3 : 1 rhythm, longest Vs. = 8.7.

14. Same. Shortly after change of rhythm from 3 : 1 (Vs. = 7.5) to 2 : 1. During stimulation rhythm became 6 : 1 (?) : 1 Vs. = 11.

15. Same. Before: rhythm 3 : 1 (1 Vs. = 7.5). During: rhythm = 5 : 1 (?) (1 Vs. = 9).

16. Same. Stimulation more rapid. Before: rhythm 3 : 1 (1 Vs. = 7.5). During: rhythm apparently 9 : 1 and 10 : 1 (longest Vs. = 14). After: rhythm becomes 3 : 1.

17. Same. Before: rhythm 5 : 1 (1 Vs. = 12.7). During: block apparently becomes complete; longest Vs. = 22, and corresponds with approximately 15½ As'.

18. Same. Stimulation more rapid. (12 stimuli and 5¾ As' in 15). Block complete. No change in V. rate.

19. Stimulation of ventricles in complete block with constant current (2 Edison-Lalande cells). Before: approximately 2 Vs'. and 10 As'. in 27.6. During: 15 Vs'. and 14 As'. in 37.2. After: V. stoppage for 73; V. rate then gradually increases, but block remains complete.

20. Same. Fibrillary contractions of ventricles.

*Experiment 8.*— June 17, 1905. Large coach dog. The first time hook was inserted, block not obtained; the second time, got partial block, then complete block without stoppage. The third time obtained similar result. The block remained partial for some time. During this time vagus stimulation stopped A., but did not apparently affect V. When rhythm



became 1 : 1 vagus stimulation stopped A., whereas V. was slowed but not stopped. After another stimulation of the vagus, A. stopped beating; V. continued to beat with slow regular rhythm and suddenly went into fibrillary contractions.

*Experiment 9.* — June 17, 1905. Small fox terrier. Hook inserted; partial block obtained, which gradually gave way to normal sequence. Strongest stimulation of both vagi had no effect upon the heart at any time. Block gradually became complete. Experiment discontinued.

*Experiment 10.* — June 18, 1905. Large cat. The first time the hook was passed block was not obtained. It was withdrawn and reinserted further posteriorly. Upon clamping obtained complete block with A. beating about one and one-half times as fast as V. V. contractions soon became feeble, and finally fibrillary contractions developed.

*Experiment 11.* — June 20, 1905. Small rabbit. Hook (short arm 6 mm. long) passed — no block. Clamp tightened — slight but distinct stoppage of V., followed by complete block from which the heart did not recover.

1. Complete block. 6 Vs'. and 33 As'. in 58.

1 a. Stimulation of vagus. Rhythm apparently 6 : 1. During stimulation both A. and V. decidedly slowed, and block seemed to become complete.

2. Same. Block apparently complete; 1 Vs. in 10.3. During: A. and V. slowed; 1 Vs. in 18.2.

*Experiment 12.* — June 21, 1905. Dachshund. Used rabbit hook (see experiment 11). The first time the hook was inserted block was not obtained. Hook withdrawn from septum and reinserted: obtained complete block at once without stoppage. *The block remained complete throughout the experiment.*

1. V. rate = 60 per minute.

2. Vagus stimulation. A. inhibited; no change in V. rate.

3. No result.

4. Rhythmic stimulation of A. Rate of stimulation about twice that of normal. A. responds accurately to stimuli; no change in V. rate.

5, 6, and 7. Same. Results similar.

8. Stimulation of V. with constant current from one Edison-Lalande cell. Rapid rhythmic contractions of V. for 50 (17 Vs'). No slowing of V. after cessation of stimulation. A. rate not affected.

9 and 10. Same. V. did not contract so frequently.

11 and 12. Rhythmic stimulation of V. with induction shocks. V. responds only occasionally.

13. Same. Rate of stimulation slower. V. responds to every other stimulus (28 Vs'). Very slight slowing of V. after cessation of stimulation, *e. g.*, before, 5 Vs'. in 34.2; after, 5 Vs'. in 40.4.

14 and 15. No result.

*Experiment 13.*—June 21, 1905. Dog. Upon inserting hook got 3:1 rhythm, which later gave way to complete block. This persisted throughout the experiment.

1. Vagus stimulation. Rhythm apparently 3:1. Partial inhibition of A.; no change in V. rate.

2. Vagus stimulation. Block complete (14 As'. to 9 Vs'.) No change in V. rate, except perhaps a slight increase.

3-6. Accelerator stimulation. Slight and independent accelerations of A. and V.

7. No result.

*Experiment 14.*—June 23, 1905. Black and tan dog. Hook (short arm 8 mm. long) used. Partial block obtained upon first attempt. Then proceeded with experiment.

1. Vagus stimulation. Block complete: 5 Vs'. to 14.5 As'. Complete inhibition of A., no change in rate of V.

2. Accelerator stimulation. Practically no effect.

3 and 4. Same. Block complete. Decided and independent accelerations of A. and V. At first slight inhibition of A.; at same time V. was accelerated.

5. Same. Rhythm 3:1. At first slight inhibition of A., and decided acceleration of V., rhythm becoming 2:1. Later, when acceleration of A. occurs, rhythm becomes 3:1, returning to 2:1 as acceleration passes off shortly after cessation of stimulation.

6. Same. Rhythm 2:1. Decided acceleration of A. and V. No change in rhythm.

7. Vagus stimulation. Rhythm 2:1. Complete inhibition of A.; decided slowing of V. (longest Vs. = 7.6). During recovery, a few 1:1, then 2:1 beats.

8. Same. Results similar.

9. Vagus stimulation. Rhythm 1:1. Complete inhibition of A.; V. stops for 47, and then beats with slow, gradually increasing, rate.

10. Clamping and unclamping. Rhythm 1:1. V. record not perfectly clear, but apparently partial block for about 8 Vs'.; then stoppage of V. for 17.

11. Vagus stimulation. Block complete. Complete inhibition of A.; no change in rate of V.

12 and 13. Accelerator stimulation. Rhythm 1:1. Decided acceleration without change in rhythm.

14. Vagus stimulation. Rhythm 1:1. Stoppage of V. for 27 when A. escapes and causes V. to beat.

15. Clamping and unclamping. Rhythm 1:1. 15 of 2:1, 13 of 3:1 when recording apparatus broke.

16. Vagus stimulation. Rhythm 2:1 (?). A. completely inhibited; V. slowed: thus, Before: 5 Vs'. in 21. During: 4 Vs'. in 23.6.

17. No result.

18. Rhythmic stimulation of V. followed by vagus stimulation. Rhythm 2 : 1. Nothing of significance.

19. Accelerator stimulation. Rhythm 2 : 1. Decided acceleration without change in rhythm.

20. Vagus stimulation. Rhythm 2 : 1. Decided inhibition of A. and V. Longest Vs. not caused by As. = 10.6. During recovery rhythm 1 : 1.

21. Record of complete block. This developed spontaneously and unwatched.

22. Rhythmic stimulation of V. Rhythm 3 : 1. Rate of stimulation that of A. V. responded to every stimulation, and resumed 3 : 1 rhythm upon cessation of stimulation. No A. extra-systoles.

23. Rhythm 3 : 1 (apparently). Vagus stimulation : inhibition of A. complete ; V. rate slightly increased (before : 3 Vs'. in 20.8 ; during : 3 Vs'. in 22). Block now complete. Rhythmic stimulation of V. with approximately the rate of A : V. responds to each of 26 stimuli. Simultaneous vagus stimulation and cessation of rhythmic stimulation : V. rate slower than before (3 Vs. in 30.3).

24. Vagus stimulation. Block complete. 1 Vs. in 7.8. Complete inhibition of A. ; no apparent change in rate of V. Rhythmic stimulation of V. with induction shocks (10 stimuli in 26.8 for 58 stimulations). V. responds to each stimulus. At cessation of rhythmic stimulation, V. rate slow and somewhat irregular ; longest Vs. = 19.2. Vagus stimulation at this time : complete inhibition of A. ; no change in V. rate.

25. Accelerator stimulation. Rhythm 3 : 1 (?). Decided acceleration of A. and V. Block became complete, V. remaining accelerated.

26 a. Vagus stimulation. Rhythm apparently 3 : 1. Complete inhibition of A : slight slowing of V.

27. Vagus stimulation. Rhythm apparently 3 : 1. Almost complete inhibition of A. ; V. rate somewhat irregular. During recovery from inhibition rhythm apparently 2 : 1 (?). Now rhythmic stimulation of V. with induction shocks, same rate as above : V. responds to each of 64 stimuli. At cessation of stimulation of V. 2 short Vs'. (about 5 each), then stoppage for 29.8. V. rate then gradually increases. Now vagus stimulation : almost complete inhibition of A., no positive change in V. rate.

28. Same. Results similar except that during recovery from inhibition the block is definitely complete.

29. Vagus stimulation. Rhythm 1 : 1. Complete inhibition of A. ; marked V. stoppage : 1st Vs. (longest) = 31.5.

30. Clamping and unclamping. Rhythm 1 : 1. Stoppage without intermediate stages : The longest Vs. (1st) not entirely recorded ; part obtained = 12.8. Followed by complete block.

31. Rhythmic stimulation of V. during recovery from No. 30. Rhythm

3 : 1. Stimulus same as that used in No. 28. To this V. did not respond. Rhythm changed to 2 : 1, then to 1 : 1.

32. Vagus stimulation during minimal rhythmic stimulation of V. Block apparently complete. Rhythmic stimulation of V.: V. does not respond to each stimulus (to 28). During vagus stimulation number of responses apparently less than before. Simultaneous cessation of both stimulations: V. rate slow and irregular, longest (3rd) = 19.6.

33. Same. Block complete. V. responds to all of 38 stimuli. Upon vagus stimulation during rhythmic stimulation of V., number of V. responses not affected. After cessation of stimulations, V. rate gradually increases: longest Vs. (1st) = 11.7. During rhythmic stimulation no A. extra-systoles.

34. Rhythmic stimulation of V. Rhythm 1 : 1. Stimuli about twice the A. rate. V. responded to each; many A. extra-systoles. At cessation of 19 stimulations 1 : 1 rhythm immediately resumed.

35. Rhythmic stimulation of V. Rhythm 2 : 1. Rate of stimulation same as before; number of stimuli 15. V. responded to each stimulus. No A. extra-systoles. After cessation of stimulation 1 of 3 : 1, then 2 : 1.

36 a. Vagus stimulation. Rhythm 2 : 1. Complete inhibition of A.; longest Vs. (1st) = 23.7. During recovery rhythm becomes 1 : 1.

37. Clamping and unclamping. Rhythm 1 : 1. (Block here produced by merely tilting clamp.) Stoppage of V. without intermediate stages of partial block. Duration of longest Vs. (1st) 24.3 +. Beginning of stoppage not obtained on tracing. Rhythm returns to 1 : 1 through 3 of 2 : 1.

38. Vagus stimulation. Rhythm 1 : 1. Complete inhibition of A.; 1st Vs. = 40, 2nd = 41.

39. Clamping and unclamping. Rhythm 1 : 1. Method same as above. Stoppage of V. without intermediate stages of partial block. 1st Vs. (longest) = 56.8, followed by long complete block.

40. Accelerator stimulation. Rhythm alternating between 2 : 1 and 3 : 1. During stimulation of accelerator, tendency toward 1 : 1. Shortly after stimulation rhythm becomes 1 : 1. Nos. 41-45 contain no facts pertaining to this research.

*Experiment 15.* — June 24, 1905. Black and tan. Hook passed, clamp tightened, obtained partial block. Then proceeded with experiment.

1. Accelerator stimulation. Rhythm 1 : 1. Very marked acceleration; rhythm remains 1 : 1.

2. Vagus stimulation, coil at 9. Rhythm 1 : 1, rate = 5 Vs'. in 15.6. Complete inhibition of A.; longest Vs. (2nd) = 10.8.

3. Same, coil at 8. Rhythm 1 : 1, rate = 5 Vs'. in 17. Complete inhibition of A.; longest Vs. (2nd) = 12.2.

4. Same, coil at 7. Rhythm 1 : 1, rate = 5 Vs'. in 18.7. Complete inhibition of A.; longest Vs. (1st and 2nd same) = 10.

5. Clamping and unclamping. Rhythm 1:1. 26 of 2:1, then complete block without stoppage.

6. Vagus stimulation. Block complete. Before: 3 Vs'. in 23.2; During: 3 Vs'. in 23.9.

7. Accelerator stimulation. Block complete. Decided and independent accelerations of A. and V.

8 a. Same. Rhythm 1:1 shortly after recovery from complete block. Marked acceleration. During maximum acceleration rhythm becomes 2:1, returning to 1:1 when A. rate diminishes.

9. Same, but some time after recovery from complete block. Same acceleration, but no change in rhythm. Heart-rate almost doubled.

10. Vagus stimulation. Rhythm 1:1. Complete inhibition of A.; longest Vs. (1st) = 44.

11. Clamping and unclamping. Rhythm 1:1. 6 of 2:1 and then complete block without stoppage.

12. Accelerator stimulation. Rhythm 3:1. Marked acceleration of A., rhythm becoming 4:1 for 4 Vs', and then block becomes complete. Later (12 a), when A. rate diminishes, rhythm returns to 3:1.

13 a-f. Effect of accelerator stimulations recorded on rapidly revolving drum. All results similar to the following: 13 d. A. rate rapidly increases through 11 Vs'. of 3:1. In these, As.-Vs. gradually increases in length: suddenly it becomes very long, and this is followed by 1 of 4:1 with very short As.-Vs. Then 3 of 3:1 with lengthening As.-Vs. Then 1 of 4:1 with short As.-Vs., followed by 1 of 4:1 with long As.-Vs. or complete block.

14. Vagus stimulation. Rhythm 1:1. Marked inhibition, but A. beats first and causes Vs. (duration 28).

15. Clamping and unclamping. Rhythm 2:1. 5 of 3:1, 6 of 4:1 then complete block with very slight stoppage; longest Vs. (1st) = 14.5.

16. Rhythm 1:1. Both vagi cut: no increase in heart-rate.

17. Vagus stimulation. Rhythm 1:1. A. escapes first and causes V. to beat in 21.

18. Clamping and unclamping. Rhythm 1:1. Apparently long period of 2:1, then complete block with A.:V. = approximately 2:1. No. V. stoppage.

19. Vagus stimulation. Rhythm 1:1. A. escapes first and causes V. to beat in 20.4.

20 and 21. Same, but stimulus stronger. Results same as above.

22. Clamping and unclamping. Rhythm 1:1. A. record not clear, but apparently block comes on from 1:1, the 1st Vs'. being but slightly longer than As.; length of Vs'. then increases very gradually, longest Vs. = 8. (REMARK. This anomalous result seems to be due to the action on V. of some constant stimulus, perhaps the pressure exerted



by the clamp. It is perhaps comparable with Gaskell's rhythm of excitation.)

23 and 24. No result.

25. Effect of vagus stimulation during rhythmic stimulation of V. in complete block. Before: 3 Vs'. and 9.8 As'. in 25.6. Rate of induction shocks 5 in 10.3. V. responds to almost every stimulus (34 Vs'). During vagus stimulation complete inhibition of A.; no change in response of V. to stimuli. Cessation of vagus stimulation: no change in V. response. Cessation of rhythmic stimulation: 1st Vs. = 12, 2nd = ?, 3rd = 12.3.

26. Rhythmic stimulation of V. Block complete. Rate of stimulation same as above. Before: 3 Vs'. and 11.8 As'. in 31.8. V. responds to stimuli (31 Vs'.) less frequently than above. Cessation of rhythmic stimulation: 1st Vs. = 15.2, 2nd and 3rd slightly longer.

27. Effect of accelerator stimulation during rhythmic stimulation of V. Block complete. Before: 2 Vs'. and 9 As'. in 24.9. Rhythmic stimulation of V.: V. responds rather infrequently. Stimulation of accelerator: A. rate increases to 5 As. in 12.4. No decided change in response of V. to rhythmic stimulation. Cessation of rhythmic stimulation: 1st Vs. = 17.5, 2nd = 18.2.

28. Same. Before, V. somewhat irregular, longest Vs. and 5.7 As'. in 15.5. Rhythmic stimulation of V.: V. does not respond to each stimulus. Stimulation of accelerator: (5 As'. in 13) no change in response of V. to stimulation. Cessation of rhythmic stimulation: 1st Vs. = 32.

*Experiment 16.*—June 26, 1905. Small fox terrier. Block was obtained in the first position occupied by the hook. Before proceeding with the experiment an infusion of salt solution was given to make up for the severe loss of blood through the puncture in the aorta.

Throughout the experiment there was a constant tendency for complete block to develop.

1. Stimulation of right vagus, coil at 9. Block complete; 4 Vs'. and 11.3 As'. in 24.7. During stimulation, A. completely inhibited; 4 Vs'. in 24.7. During recovery: 1 of 1:1 and then 2:1.

2. Stimulation of right vagus, coil at 9. Rhythm 2:1. During stimulation: complete inhibition of A.; 1st Vs. = 7.2, 2nd = 7, 3rd = 8.1, 4th = 8.7. (During complete block at beginning of experiment 1, Vs. = 6.) During recovery rhythm 1:1 for some time.

3. Shows 2:1 rhythm developing from preceding 1:1. A. rate now about same as before above stimulation.

4. Spontaneous block. Developed gradually, preceded by slight stoppage: 1st Vs. on tracing (beginning not recorded) = 7.4.

5. Stimulation of right vagus, coil at 9. Block complete: 13.7 As'. in 4 Vs', 1 Vs. in 6.8. During stimulation: complete inhibition of A., no



change in V. rate. During recovery, rhythm 1 : 1; A. rate slower than before stimulation, but V. rate markedly increased, more than doubled.

6. Later. When A. rate becomes about the same as before stimulation, rhythm becomes 2 : 1.

7. Immediately after onset of spontaneous complete block. Successive Vs' = 8.2, 9, 10.2, 10.9, 10.9, 9.5. Later, when rate becomes constant, 1 Vs. = 7. Preceding stages not noted.

8. Vagus stimulation. Block complete, 4 Vs' in 14.2 As'. During stimulation, complete inhibition of A. (*i. e.*, no spontaneous As'), V. rate not changed; the first 3 Vs' apparently cause As', *i. e.*, reversed rhythm. Later, as A. rate increases, heart-beat is determined by A. and rhythm becomes 1 : 1.

9. Later. Rhythm 2 : 1.

10. Later, the above 2 : 1 rhythm (1 Vs. in 4) changes into complete block spontaneously: the duration of the 1st Vs' = 8.7, 9.4, 10.6, 10.5, 10.7. Later, see No. 11.

11. Vagus stimulation, coil at 9. Block complete. Before: 11 As' in 3 Vs'; 1 Vs. in 7.2. During: complete inhibition of A., V. rate not changed. 1st Vs. reverses rhythm. After: short period of 1 : 1, then alternating 2 : 1 and 1 : 1.

12. Later. Block complete; 4 Vs' in 14 As'; 1 Vs. in 7.3.

13. Stimulation of vagus, coil at 9. Block complete. Complete inhibition of A., no change in V. rate. After: 2 of 1 : 1, 19 of 2 : 1, short period of alternating 2 : 1 and 3 : 1, 3 : 1, and finally complete block without stoppage.

14. Stimulation of vagus, coil at 9, immediately after development of complete block. Complete inhibition of A., no change in V. rate. During recovery: 27 of 1 : 1, then 2 : 1.

15. Vagus stimulation. Rhythm 2 : 1. 1 Vs. in 3.9. During: successive Vs' = 10, 10.6, 10.7 (retrograde impulse), 10.2 (retrograde impulse), 10 (retrograde impulse) 9.5, 5.9 (caused by As.), and somewhat later rhythm 1 : 1.

16. Vagus stimulation, coil at 9. Rhythm 1 : 1. Before: 2 Vs' in 4.5. During: successive Vs' = 8.3, 13, 14.5, 12.4 (possibly caused by As.), 12.3, 8.4 (caused by As.); then 1 : 1.

17. Same; stimulation stronger (coil at 8). Before: 2 Vs' in 4.5. During: successive Vs' = 7.9, 15.2, 17.5 (possibly retrograde impulse), 17.1 (caused by As.?), 7.2 (caused by As.); then 1 : 1.

18. Same; stimulation stronger (coil at 7). Before: 2 Vs' in 4.2. During: successive Vs' = 7.4, 11.1 (caused by As.?), 17.3 (retrograde impulse), 16.2 (caused by As.?), 9.5 (caused by As.); then 1 : 1.

19. Clamping and unclamping. Rhythm 1 : 1. Before: 2 Vs' in 4. During: 20 of 2 : 1 (1 Vs. in 4.2), then complete block with successive Vs' = 4.7 (?), 7.1, 6.3, 6.5, 6.3, 6.4.

20. Now vagus stimulation. Before: 3 Vs'. in 18.8, and 9 As'. in 3 Vs'. = 3:1. During: complete inhibition of A.; 3 Vs'. in 19.1. Tracing indistinct during recovery.

21. Vagus stimulation, coil at 7. Rhythm 2:1. Before: 3 Vs'. in 12.2. During: complete inhibition of A.; successive Vs'. = 8.1, 8.1, 8.2, 7.8 (retrograde impulse?), 7.6, then 1:1.

22-27. Stimulation of vagus, but for unknown reason inhibition of A. was incomplete. In this interval *block* became *permanently complete*.

28-30. Rhythmic stimulation of V. with induction shocks. V. responds to stimuli occasionally.

31. Vagus stimulation during rhythmic stimulation of V. During vagus stimulation V. does not respond to stimuli as well as in No. 30.

32. Same. Before: 1 Vs. in 7.6. During vagus stimulation: inhibition of A. complete; perhaps a slight improvement in response of V. to rhythmic stimulation. After: 1 Vs. in 9.

33-35. Same. During vagus stimulation: inhibition of A. always complete, but V. shows absolutely no change in its response to the rhythmic stimuli.

36. Same. Result the same. Period of rhythmic stimulation = 300 (approximately 163 stimuli). After rhythmic stimulation, successive Vs'. = 8.2, 68.8, 32.6, 26.8, 25.4. V. rate then gradually increases until same as before.

37. Same. Results similar. Duration of period of rhythmic stimulation shorter (79 stimuli). After rhythmic stimulation, successive Vs'. = 9, 22.5, 46, 33.3.

38. Same.

39. Same. Stimuli stronger. Similar results. V. responds to all of 218 stimuli at rate of 150 per minute. After: longest Vs. (3rd) = 77.

40. Same. Period of rhythmic stimulation shorter (24). After: longest Vs. (4th) = 14.

41. Same. Results similar.

42. Same, but rate of rhythmic stimulations increased. Fibrillary contractions of V.

*Experiment 17.*—June 28, 1905. Small fox terrier. Partial block obtained with the first adjustment of the clamp. Then proceeded with the experiment.

1. Rhythm 1:1.

2-7. Vagus stimulation with increasing strengths. Rhythm 1:1. As. always causes Vs.

8. Vagus stimulation, coil at 6. Rhythm 1:1. Rate = 3 Vs'. in 8.3. As. causes Vs. for about 7 cycles. Here 1 Vs. = 6.8. Suddenly Vs. becomes longer (1 Vs. = 8.6); this Vs. was not caused by As.

9. No result.

10. Stoppage of V. while adjusting clamp. Before: rhythm 1:1. Rate = ?. Beginning of stoppage not recorded. 1st full Vs. recorded = 15.2 (about 5 As'. in same time). V. rate increases through 8 Vs'. and then becomes practically constant with 1 Vs. in 9. Block complete.

11. Later. Block probably complete. V. irregular.

12 and 13. Rhythmic stimulation of V. with induction shocks. Rhythm probably 3:1. Nothing of significance.

14. Stoppage of V. while adjusting clamp. Beginning of stoppage not recorded. 1st full Vs. on tracing = 46. 8 As'. in the same time.

Heart now began to fail, so experiment concluded.

*Experiment 18.* — June 29, 1905. Large fox terrier. Partial block obtained with the first adjustment of the clamp, then proceeded with the experiment.

1. Vagus stimulation, coil at 9. Rhythm 1:1. Before: 9 Vs'. in 20.5. During: successive Vs'. = 8.2, 15.1, 15.7 (followed by As.), 14.5 (followed by As.), etc.

2. Same, coil at 8. Result practically identical. Longest Vs. = 15.9.

3. Same, coil at 7. Result practically identical. Longest Vs. = 15.8.

4. Clamping and unclamping. Rhythm 1:1. Got 3:1 rhythm through 21 of 2:1, but not complete block.

5 and 6. Vagus stimulation. Rhythm 1:1. Each Vs. caused by As. Longest Vs. = 8.9.

7. Clamping and unclamping. Rhythm 1:1. 6 of 2:1 (?), followed by complete block without preliminary V. slowing. V. rate is here very fast, approximately 1 for every 2 As'. Appears as though V. were responding to a constant abnormal stimulus.

8 and 9. Rhythmic stimulation of V. with induction shocks. Block complete. V. responds but rarely. No V. slowing after cessation of stimulation.

10. Vagus stimulation, coil at 7. Block complete. Almost complete inhibition of A.; practically no change in V. rate.

11. Rhythmic stimulation of V. with induction shocks. Block complete. Before: 3 Vs'. and 7.5 As'. in 18.3. During: V. responds more frequently than in No. 9. Stimulation lasts 175. After: 1st Vs. = 21.3 (9 As'. in same time); V. rate then increases gradually.

12 and 13. Same, but stimuli stronger. Results practically the same.

14. Vagus stimulation, coil at 7. Rhythm apparently 3:1. Before: successive Vs'. = 7.2, 7.2, 7.2, 7.2. During: the only As'. are caused by Vs'. Successive Vs'. = 6.8, 7, 6.9, 6.8.

15. Same, shortly after rhythm becomes 2:1. Before: 3 Vs'. in 14.5. During: the only As'. are caused by Vs'.; successive Vs'. = 7.3, 7.3, 7.4.

16. Same, coil at 6. Later in 2:1 rhythm. Before: 3 Vs'. in 15.4.

During: successive Vs' = 7.8, 7.7 (retrograde As.), 6.6 (retrograde As.), 8, 8.2.

17. No result.

18. Same; rhythm alternate 2:1 and 1:1. During: successive Vs' = 7.9 (retrograde As.), 8.7 (retrograde As.), 9.3, 10.3, 10.2.

19. Same; rhythm 1:1. Before: 3 Vs' in 7.7. During: successive Vs' = 8.3 (retrograde As.), 10.9 (same), 10.9 (progressive As.), 12.4 (same), 11.9 (same).

20. Same. Fresh part of nerve stimulated. Rhythm 1:1. Result practically the same.

21. Rhythmic stimulation of A. followed by vagus stimulation, coil at 6. Rhythm 1:1. Before: 6 As' in 15.5. During: 6 As' in 9.1; A. responds to stimuli perfectly, V. somewhat irregularly but on the whole rate somewhat increased. During vagus stimulation: successive Vs' = 7.6, 3.3 (progressive As.), 5.3 (same), 11.3 (same), 14.2 (same).

22. Same. Rhythmic stimuli stronger. Before: 2 Vs' in 5.8. During: 4 As' in 6.1; A. responds perfectly; V. responds to every other As., therefore V. rate not changed. During vagus stimulation: successive Vs' = 7.8, 4.8 (simultaneous As.), 8.1 (retrograde As.), 11.3, 12.1, 12.5.

23. Clamping and unclamping. Rhythm 1:1. 2:1 and 3:1 rhythms, but not complete block.

23 a. Vagus stimulation, coil at 6, while rhythm 2:1. Before: 2 Vs' in 11.4. During: successive Vs' = 9.2 (progressive As.), 9 (same), 10.9, 9.2.

24. Same, electrodes on fresh part of nerve. Rhythm 1:1. Before: 4 Vs' in 13. During: successive Vs' = 10 (retrograde As.), 11.2 (progressive As.), 10.3 (same), 10.8 (same).

25. Clamping and unclamping. Rhythm 1:1. 4 Vs' in 14.2. 3 of 2:1 followed by complete block without preliminary stoppage of V. V. rate in complete block unusually rapid: 4 Vs' and 7 As' in 21.8.

26. Rhythmic stimulation of V. with induction shocks. Block complete. Before: 3 Vs' and 6.7 As' in 20.9. During: V. responds to stimuli quite irregularly, but, on the whole, rate is faster than before. After: successive Vs' = 8.4, 8.4, 8.6, 8.6, 8.2.

27-29. Same. Results similar.

30. Same, stimuli stronger and slower. Before: 3 Vs' in 22.7. During: V. responds to almost all of 44 stimuli (5 in 13.5). After: successive Vs' = 9.3, 5.9, 11, 9.9, 10.2.

(NOTE. Tracings were not continued until constant rate obtained. This is, however, always given under "Before" in the procedure that follows.)

31. Record not clear.

32. Same, but stimuli stronger. Before: 3 Vs'. and 6.5 As'. in 22.8. During: rate of stimulation about same as in No. 30; V. responds to almost all of 100 stimuli. After: successive Vs'. = 13.5, 12, 12, 11.2, 12.4. Experiment concluded.

(REMARK. — Note in this experiment the short V. stoppages during vagus stimulation, upon clamping (none) and after rhythmic stimulation of V. Compare with other experiments.)

*Experiment 19.* — June 30, 1905. Dog. Partial block obtained with the first adjustment of the clamp. Then proceeded with the experiment.

1. Vagus stimulation, coil at 9. Rhythm 1 : 1. Before: 5 Vs'. in 11. During: successive Vs'. = 46, 19.3 (caused by As.).

2. V. stoppage while adjusting clamp. Rhythm 1 : 1. Before: 5 Vs'. in 10. During: successive Vs'. = 18.5, 12.5, 4.8, 10.4, 7.2, 7.2, 15.2, 21.9, 21.2, 13.6.

3. Later, during return to normal: 3 of 2 : 1, then normal 1 : 1.

4. Vagus stimulation, coil at 9. Rhythm 1 : 1. V. slowed about  $\frac{1}{2}$ .

5. Same. Before: 5 Vs'. in 9.9. During: 1st Vs. = 16.8. Other Vs'. about 5.7; probably caused by As'.

6. Same. Before: 5 Vs'. in 11.3. During: successive Vs'. = 8.8 (progressive As.), 7.9 (retrogressive As.), 7.5 (same).

7. Same; coil at 8. Before: 5 Vs'. in 13.1. During: 1st 6 Vs'. all approximately 6; then 1 Vs. = 10.1, followed by a number like the first.

8. Same, coil at 7. Result similar.

9. Effect of adjusting clamp. Rhythm 1 : 1. Before: 5 Vs'. in 14.8. During: 1st obtained .4 short Vs'. which bear no apparent relation to As'; duration = 3.8, 3, 3.4, 3.7. Then longer Vs'. , most of which seem to bear a relation to As'. — 11.6, 7.8, 7.6, 9.2, 9.3, 10.4, 5.8; then about same length for some time.

10. Later. Rhythm 2 : 1.

11. Later. Rhythm 1 : 1.

12. Vagus stimulation, coil at 7. Rhythm 1 : 1. Before: 5 Vs'. in 10.5. During: apparently complete inhibition of A.; 1st Vs. = 31, 2nd = 20.2.

13. Same. Before: 5 Vs'. in 14.4. During: successive Vs'. = 34.4, 13, 6.9 (caused by As.).

14. Clamping and unclamping. Rhythm 1 : 1; 5 Vs'. in 14.2. Before block: quite a marked increase in rate, then for a short while some irregularity with 1 Vs. to about  $1\frac{1}{2}$  As'. (possibly a 2 : 1 rhythm with extrasystoles), 4 of 3 : 1, 4 of 4 : 1, and finally complete block in which successive Vs'. = 7.5, 7.8, 10.2, 9.3.

15. Vagus stimulation, coil at 7. Rhythm 2 : 1. Before: 5 Vs'. in 19.2. During: inhibition of A. apparently complete; successive Vs'. = 6.6, 6.1, 6.3, 6.8.



16. Same. Rhythm 1:1. Before: 5 Vs'. in 13.4. During: 1st Vs. = 14.5, and somewhat later another lasting 15.8 (caused by As.).

17. Same. Before: 5 Vs'. in 13.8. During: considerable irregularity; longest Vs'. not caused by As'. = 11.4, 11.2, 11.8.

18. Block while adjusting clamp. Rhythm 1:1. Before: 5 Vs'. in 11.2. Block came on suddenly; successive Vs' = 3.4, 4, 4.6, 5.5, 12.7, 6.2, 4.7, 12.9, 8.8, 4.9. (This change in V. rate occurred while the block was complete.)

19. Block still complete. V. rate still irregular.

20. Later. Block still complete. At a time when the V. rate was quite slow V. suddenly developed a fairly regular and rapid rate: 5 Vs'. and 9 As'. in 13.4.

21. Later. Block still complete. Intervals between Vs'. again long and irregular. Vagus stimulation: A. completely inhibited; no positive effect upon V.

22. Rhythmic stimulation of V. with induction shocks. Block complete. Before: 3 Vs'. and 10 As'. in 15.3. During: V. responds irregularly but usually to every other stimulus. After: successive Vs'. = 10, 11.5, 10.2, 9.5, 9.4, 9.1.

23. Same, but stimuli stronger. V. responds more frequently. V. slowing greater than after No. 22.

24. Same, but stimulation slower. Result similar.

25. Vagus stimulation, coil at 7. Block complete. Complete inhibition of A.; no positive effect on V.

26. Rhythmic stimulation of V. Rate of stimulation slower, and continued over a longer time (100 stimuli). After: V. rate slow and quite irregular.

(REMARK.— Note the irregularity of V. in all forms of stoppage. These irregularities make it difficult to determine satisfactorily the maximum cycles of inhibition and of stoppage. Some of the irregularities appear much like stoppages in the midst of complete block.)

*Experiment 20.*— October 28, 1905. Small shepherd. With the second adjustment of the clamp obtained complete block without stoppage, but there probably were some intermediate stages. Block remained complete for some time. When the rhythm became 2:1, tested the effect of stimulating the right auricle with rhythmic unipolar induction shocks. In the most successful trials got typical stoppage of the ventricles. Thus in one trial A. rate = 32 in 10 seconds, rhythm 2:1. Rate of stimulation 42 in 10 seconds. A. responded to all stimuli: 1st Vs. = 19 As', 2nd = 8, — then rate increased somewhat irregularly. Could not determine whether Vs'. were caused by As'. Often during rhythmic stimulation the rhythm remained 2:1. Rarely the rhythm became 3:1 during rhythmic stimulation. When this happened it was possible to obtain stoppage by



stimulation of the auricles after tightening the clamp, but not by enough to increase the degree of block. Later stimulation of the vagus stopped V. about twice as long as it was possible to stop it by increasing the auricular rate.

*Experiment 21.* — October 30, 1905. Fox terrier. With first adjustment of clamp obtained complete block with stoppage. Clamp beautifully placed; with a few turns of the nut any degree of block could be obtained with great precision. Upon clamping always obtained 2 : 1, followed by complete block, preceded by stoppage usually lasting about 13 to 15 seconds (longest Vs'. first). Often by adjusting the clamp carefully, got complete block with stoppage lasting, in the best trials, 10 seconds, from 1 : 1 rhythm (A. rate = 20 in 10 seconds) by stimulating A. at the rate of 38 in 10 seconds.

At one time in the experiment spontaneous changes in the order: complete block, a few of 2 : 1, 1 or 2 of 1 : 1, and then stoppage occurred successively many times.

Found that when acceleration of the auricles while the rhythm was 1 : 1 would result in stoppage, the same stoppage could be obtained by causing A. to fibrillate. The 1 : 1 rhythm was resumed immediately upon recovery of A.

# TEMPERATURE AND HEART ACTIVITY WITH SPECIAL REFERENCE TO THE HEAT STANDSTILL OF THE HEART.

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THE influence of temperature variations on the activity of the heart has been studied in great detail both in the cold-blooded and the warm-blooded vertebrates.<sup>1</sup> Within the physiological limits for the heart the rate of the beats stands in direct relation to the temperature, so that the higher the temperature the more rapid the rhythm, except for temperature near the upper physiological limit. This is true both for cold-blooded and warm-blooded vertebrates. The influence of temperature variations on the strength of the beats appears to be more complex. In the very rapid rhythm produced by the temperature near the upper physiological limit the beats are always diminutive both in the frog and the mammal, but within the range of a few degrees above and below the physiological optimum of the heart an increase in the temperature augments not only the rate but also the strength of the beats. The beat of the frog's heart may in some cases be as strong at the temperature of 0° or 4° C. as at 10° or 20° C.

A point of particular interest is the standstill in diastole of the heart of cold-blooded vertebrates at the temperature of 32° to 40° C. In this condition the heart remains excitable to direct stimulation, and on lowering the temperature the rhythm reappears. Further rise in temperature, or a long continuation of this temperature, sends

<sup>1</sup> For the literature the reader is referred to the papers by STEWART: *Journal of physiology*, 1892, xiii, p. 59; LANGENDORFF: *Archiv für die gesammte Physiologie*, 1897, lxvi, p. 355; *Ergebnisse der Physiologie, Biophysik*, 1903, Q. 2, p. 517; HERLITZKA: *Zeitschrift für allgemeine Physiologie*, 1905, v, p. 265; SNYDER: *University of California publications, physiology*, 1905, ii, p. 125; VINTSCHGAU: *Archiv für die gesammte Physiologie*, 1904, cii, p. 185.

the heart into heat rigor. Various explanations of the nature of this heat standstill have been given. Aristow<sup>1</sup> thought the standstill was due to paralysis of the motor ganglia of the heart. Cyon<sup>2</sup> suggested that the standstill was brought about by heat stimulation of the inhibitory nerve endings in the heart; but the facts that the standstill may be maintained till the heart dies or goes into rigor, and that the standstill is just as readily obtained in atropinized hearts, seem to militate against Cyon's view. Stewart<sup>3</sup> concludes that "the standstill is due to loss, not of contractility, but of spontaneous contractility of the heart muscle." This would necessarily be the true explanation of the standstill of the sinus, in case the automatism resides in the heart muscle itself, but it is not strictly true of the heat standstill of the ventricle or the auricles. Stewart found that when the temperature of the whole heart is raised the different parts of the heart become quiescent in diastole in the following order: ventricle, auricles, sinus. Thus the ventricle will be found in complete quiescence while the sinus and the auricles still continue to beat. Now, under normal conditions the ventricle beats in response to impulses reaching it from the auricles, and the auricles beat in response to impulses reaching it from the sinus. Martin<sup>4</sup> claims that the ventricle of the tortoise does not beat automatically under the conditions of normal life, and we have yet to show that this is not also true for the frog. The normal ventricular rhythm of the frog is thus due, not to spontaneous activity, be it of its muscular or its nervous tissue, but to impulses or "waves of contraction" reaching it from the auricles. The heat standstill of the ventricle must therefore be due to failure of these stimuli to reach the ventricle, or to such a change in the ventricle that it can no longer respond to these impulses from the auricles, although they continue to reach it. The same conditions must necessarily obtain in the standstill of the auricles while the sinus continues in rhythm; for although the auricles exhibit a greater degree of automatism than the ventricle, yet under normal conditions the auricles are the servitors of the sinus. The statements in the literature do not enable us to decide whether the ventricular and auricular standstill is due to loss of excitability of these parts or to failure of conduction at the sinus-auricular and

<sup>1</sup> ARISTOW: *Archiv für Physiologie*, 1879, p. 198.

<sup>2</sup> V. CYON: *Sächsische Berichte*, 1866, p. 302; LUDWIG'S *Arbeiten*, 1867, p. 118.

<sup>3</sup> STEWART: *Loc. cit.*

<sup>4</sup> MARTIN: *This journal*, 1904, xi, p. 103.

the auriculo-ventricular junctions. This can probably be put to the crucial test by local warming of these junctions.

According to Langendorff, this heat standstill in diastole cannot be obtained in the mammalian heart, the heart of warm-blooded vertebrates continuing its rhythm up to the point where the heat rigor sets in.<sup>1</sup> According to Luschinger,<sup>2</sup> however, the rhythmically pulsating veins in the wings of the bat become quiescent in diastole at a temperature slightly lower than that producing heat rigor. The pulsating veins of this mammal thus exhibit the same reaction to rising temperature as the heart of cold-blooded vertebrates. And Herlitzka<sup>3</sup> has recently shown that the typical heat standstill may be obtained in the mammalian heart perfused with Locke's solution.

### I. EXPERIMENTAL METHODS.

The series of experiments about to be described were undertaken, not so much for the purpose of determining whether variations of temperature act on the invertebrate heart in the same manner as on that of the vertebrates, but rather to eliminate a possible source of error in my work on the point of action of drugs in the heart. It seemed possible that some of the effects of different drugs and salts in solution might be due to slight difference in temperature of these solutions. It was therefore desirable to determine in what way slight variations of temperature affect, first, the ganglion, secondly the heart-muscle, thirdly, the nerve trunks of the intrinsic nervous plexus. This led to the discovery that *the influence of temperature variations on the heart muscle does not run parallel with that on the heart ganglion and the intrinsic nervous plexus*. In other words, I was able to make an analysis of the action of heat and cold on the heart not yet made in the vertebrates. Experiments with high temperatures led to the further discovery that *the heart muscle ceases to respond to the impulses from the ganglion, remains in diastole, and relaxes its tonus at a point where the ganglion still continues its activity and the nerves retain their conductivity*. This point seemed new, and an extended series of experiments were carried out to exclude every possible error in the results. These results were not to have been expected from what we know of the influence of high temperatures

<sup>1</sup> LANGENDORFF: *Loc. cit.*

<sup>2</sup> LUSCHINGER: *Archiv für die gesammte Physiologie*, 1881, xxvi, p. 445.

<sup>3</sup> HERLITZKA: *Loc. cit.*

in the vertebrate heart. In fact, some of the results of Langendorff on the mammal and Stewart on the frog would seem to indicate that this peculiar relation may not obtain in the heart of higher forms. These points will be referred to later.

The three methods of preparing the *Limulus* heart for these experiments are shown diagrammatically in Fig. 1. They were briefly described in my preliminary note on the point of action of drugs on the heart.<sup>1</sup> For studying the effects of temperature on the ganglion alone, the ganglion is completely isolated from the heart, save in the two first heart segments, which serve as an indicator of the activity of the ganglion. This isolated ganglion can be put under any conditions of temperature, while that of the muscle indicator remains constant at any temperature desired. The activity of the muscle of the first two segments of the heart depends on the ganglion of the middle portion;<sup>2</sup> the leaving of the nerve cord intact on the anterior end does therefore not invalidate the results. The ideal preparation would be the isolation of the entire dorsal nerve plexus of the extirpated part of the heart in Diagram I, and leaving it in connection with the reacting muscular portion; for

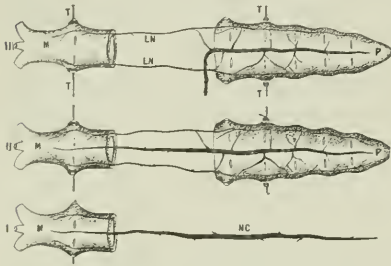


FIGURE 1. — Diagrams to illustrate the preparation of the *Limulus* heart for studying the influence of temperature on the activity of the muscular and the nervous tissue. I. Nerve cord isolated from the muscle posteriorly, the muscle and lateral nerves transected in second segment. II. Heart muscle removed in middle third of heart. Nerve cord and lateral nerves intact. Both ends of heart prepared for graphic registration. III. Heart muscle removed from middle third of heart. Nerve cord removed from anterior end, leaving lateral nerves intact. *LN*, lateral nerves; *NC*, nerve cord; *M*, musculature of anterior end of heart; *P*, posterior end of heart; *T*, threads for fixing the reacting portions in the cylinders and to the levers.

the lesion of the lateral nerves severs many connections between the ganglion and the muscle, thus rendering the contractions weaker. But this dissection is very difficult. I have succeeded only in a few cases in isolating the nerve cord together with the lateral nerves and all of their main connections. The necessary weakening of the beats in preparation I does not really affect our results. It is not necessary even to isolate the ganglion on the middle and pos-

<sup>1</sup> CARLSON: *Science*, 1904, xx, p. 684.

<sup>2</sup> CARLSON: *This journal*, 1904, xii, p. 67; p. 471.



terior portion of the heart, as the *Limulus* heart is so long that this portion of it may be warmed or cooled for considerable periods before the temperature is equalized by conduction in the tissue so that the anterior end, surrounded by different temperature, is affected. Experiments made in this manner gave results to all appearance identical with those obtained on preparation J. The possibility suggested itself to me that when the whole posterior end of the heart is subjected to temperature variations, the direct action on the ganglion might be influenced by action on possible local reflex mechanisms; but even if this actually is the case, it does not appear plainly in the results. The advantage of the almost entirely complete nervous connections between the anterior and the posterior ends with the facility of temperature conduction lessened may be gotten by removing the heart muscle for a distance of one or two centimetres in the second and third segments, leaving the lateral nerves and the nerve cord intact, as represented in Fig. 1, Diagram II. This dissection was frequently used. It is not difficult to prepare in case of the largest hearts.

The influence of temperature variations on the heart muscle apart from that on the ganglion can in reality be determined on the anterior end of the heart prepared as shown in the two diagrams just described; for although the nerve cord is left on the first two segments, that portion of the cord contains so few ganglion cells that it reacts almost like one of the lateral nerves, which contains no ganglion cells. But in order to exclude even the possibility of complications, owing to the presence of the ganglion on the reacting portion, the preparation shown in Diagram III, Fig. 1, was used exclusively. In this preparation the nerve cord is transected in the third segment and the anterior portion completely removed. The posterior portion is isolated from the muscle down to the fourth segment, but left in connection with the rest of the nerve plexus. The lateral nerves are left intact, but the heart muscle is completely removed for the distance of two segments including the third, half of the second, and half of the fourth, respectively, as indicated in the diagram. The rhythm of the anterior end of the heart in this preparation depends on the impulses reaching it through the lateral nerves. The anterior end may be warmed or cooled with no possibility of heat conduction back through the tiny lateral nerves in sufficient amount to alter the temperature of the posterior end of the heart and the plasma or seawater surrounding it.



Many experiments were made on the effect of temperature variations of the heart muscle, making use of this preparation, no graphic record being taken of the rhythm of the posterior end. In order to make it absolutely certain that changes in the rhythm of the anterior end concomitant with the temperature changes were due to action of the temperature on that end, and not brought about, at least in part, by the stimulation of afferent paths of local reflex mechanisms, thus altering the activity of the ganglion on the posterior portion, I found it necessary to take simultaneous records of the two ends. The recording lever was usually connected with the fifth segment of the posterior portion by silk threads secured to the suspensory ligaments in the manner shown in Diagram III. The preparation III, used for simultaneous tracings from two ends, lends itself admirably to the demonstration of the fact that the muscle ceases to respond to the nervous impulses, and relaxes its tonus before the ganglion ceases its activity and the nerves cease to conduct.

So far as I can see, there is no way of preparing even this very accommodating heart so that the influence of temperature on the muscle may be studied apart from that on the motor nerve endings. I have shown elsewhere that there are no inhibitory nerves to the muscle of this heart.<sup>1</sup> But the effect of different degrees of temperature on the conductivity and the excitability of the nerve fibres themselves apart from that on the ganglion and the muscle and nerve endings can be determined on preparation III by letting a loop of the isolated lateral nerves dip into a reservoir or vessel separated from those containing the anterior and the posterior ends respectively (Fig. 2, *E*). The temperature of this loop of the nerves can thus be varied independently, and accurate results obtained by taking simultaneous graphic records of the rhythm of the two ends of the heart. This procedure gives the influence on the power of conduction of the nerves as judged by the strength of the impulse after having passed through the cooled or warmed area, but it does not give results sufficiently accurate to reveal small variations in the rate of conduction.

The temperature variations of the different parts of the heart were brought about by varying the temperature of the plasma or sea-water surrounding these parts. After some preliminary trials I found the following apparatus and devices convenient and satisfactory. The arrangements for the experiments are illustrated in Figs. 2 and 3.

<sup>1</sup> CARLSON: This journal, 1905, xiii, p. 217.

The scheme for the experiments involving graphic records of only one end of the heart is that represented in Fig. 2. As reservoirs for the reception of the two ends of the heart I made use of glass cylinders of convenient size. These were closed at the bottom by rubber stoppers, through which passed two glass tubes, the inlet and outlet tubes respectively. The inlet tubes of the two cylinders were connected to a T-piece, and this again to a funnel secured to a stand. By means of this device plasma or sea-water at any desired temperature can be supplied to either cylinder at will. The inlet tube extends

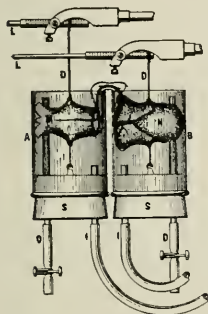


FIGURE 3. — Diagram to illustrate the arrangement of apparatus and heart preparation for obtaining tracings from both ends of the heart under conditions allowing independent variations of the temperature of either end. The heart preparation represented is II, Fig. 1. *N*, posterior end of heart; other letters same as in Fig. 2.

only a little way from the bottom of the cylinders, but the outflow tube has to extend almost to the top, in case graphic records are taken from the portion of the heart which it contains. Sometimes the fluid used for bathing the preparations was allowed to escape over the edge of the cylinders. If the level of the liquid does not remain the same throughout an experiment, the tension of the suspended heart portion on the recording lever varies, thus

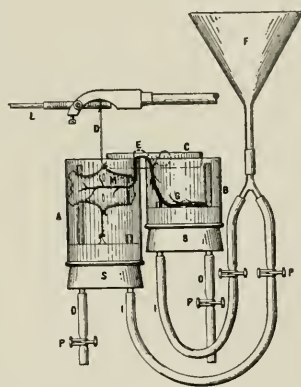


FIGURE 2. — Diagram to illustrate the arrangement of apparatus and heart preparation for studying the influence of temperature variations on the heart ganglion apart from that on the heart muscle. *A*, cylinder for the reception of the reacting portion or first two heart segments; *B*, cylinder for the reception of the posterior portion of the nerve cord; *C*, cylinder used in determining the effect of temperature variations on conduction; *D*, thread connecting the reacting segments with the lever; *E*, dotted line representing loop of the nerve cord or lateral nerves dipping into cylinder *C*, for the purpose of local variation of the temperature; *F*, funnel; *G*, ganglion of the posterior two-thirds of the heart; *I*, inflow tubes to the cylinders; *L*, recording lever; *M*, reacting portion of the heart, first two segments; *O*, outflow tubes from the cylinders; *P*, pinchcocks; *S*, rubber stoppers.

introducing alterations in the abscissa on the records and apparent alteration in tonus. This device is, of course, not necessary when no graphic records are taken from the portion subjected to the test,

as, for example, when the temperature variations are confined to the isolated ganglion. The fact that it is necessary to have the fluid at the same level in case tracings are taken, makes it impossible to alter the temperature of the preparations as quickly as could otherwise be done, since it takes an appreciable time for the incoming plasma to mix with or rather expel the fluid already in the cylinder.

The reacting portion was secured in the cylinder by means of a glass or platinum hook fixed in the stopper and by another thread to the recording lever, as shown in the diagram in Fig. 2. The lateral nerves or the nerve cord connecting the two ends of the heart were allowed to hang over the edge of the cylinder, as shown in the diagram, care being taken that they were not pressed or put on the stretch. The portion extending above the liquid in the two cylinders was protected from evaporation by an inverted watch crystal or a piece of moistened filter paper.

In the experiments on the influence of temperature on the conductivity of the nerves, a loop of the isolated lateral nerves was allowed to dip into the third cylinder in the manner indicated by the dotted line at *E*, Fig. 2.

When graphic records were taken from both ends of the heart, the posterior portion was secured in the cylinder and to the lever in the same manner as the anterior end. The recording lever for the posterior end was arranged below and slightly to the side of the lever for the anterior end, as shown in Fig. 3. Both levers recorded by upward displacement. By this arrangement the abscissa of the two records must be far enough apart to allow for the excursions of the lower lever. By arranging the second lever for recording by downward excursions, the two abscissæ could have been more closely approximated, but I found the former method more convenient and sufficiently accurate for the present purpose.

It was not within the scope of the present inquiry to test the influence of temperature for long periods, hence the cylinders were not provided with jackets for maintaining constant temperature for longer periods. For our present purpose it sufficed to let the cooled or heated plasma flow through the cylinders at a slow rate or at frequent intervals. Sea-water can be used instead of blood plasma for bathing the heart, as the sea-water is almost as neutral in its action on the heart as the plasma. In these experiments sea-water was used as well as plasma, both yielding the same results.

## II. THE INFLUENCE OF TEMPERATURE ON THE HEART MUSCLE.

The optimum temperature of the heart muscle in *Limulus* lies between  $10^{\circ}$  and  $14^{\circ}$  C. Within these limits the strength of the contractions is usually not subject to great variations, but raising the temperature above  $14^{\circ}$  or lowering it below  $10^{\circ}$  diminishes both excitability and contractility. This optimum temperature probably corresponds to the temperature of the sea-water of the animal's habitat, except when in the breeding season it seeks more shallow water.

At temperatures higher than  $14^{\circ}$  or  $16^{\circ}$  C. the strength gradually diminishes till the beats cease completely at the temperature of  $32^{\circ}$  C. This cessation of the rhythm is in diastolic condition of the preparation. It is invariably accompanied by tonus relaxation. At this temperature the muscle has not lost its excitability and contractility, for it will contract on direct stimulation, although it cannot respond to the normal nervous impulses reaching it from the ganglion. The muscle remains in this quiescent and relaxed condition as its temperature is raised from  $32^{\circ}$  to  $47^{\circ}$  or  $50^{\circ}$  C., when tonus contraction or heat rigor sets in. The temperature at which the contraction of the muscle ceases is sometimes lower than  $32^{\circ}$  C., depending on the condition of the reacting portion. Reacting portions in poor condition, as towards the end of a long series of experiments, would sometimes cease to contract when the temperature reached  $25^{\circ}$  to  $28^{\circ}$  C. This is exactly what Stewart found in case of the fatigued frog's ventricle.

The cessation is an actual quiescence. Not only does a light recording lever fail to register any contractions, but no sign of contraction can be made out even by the aid of a strong lens. Furthermore, the relaxation of or loss of tonus is greater than that under any conditions when the muscle is in rhythmical activity. There is therefore no doubt that we have in the *Limulus* heart a heat standstill in diastole similar to that in the heart of cold-blooded vertebrates. It is interesting to note that this heat standstill appears also at the same degree of temperature in the frog and in *Limulus*, in the normal heart at about  $32^{\circ}$  C.

On lowering the temperature of the muscle the rhythm returns promptly, just as in the frog's heart. After heat standstill for several minutes, the rhythm that appears on cooling the preparation is

usually more vigorous than the original. The failure of the muscle to contract at this high temperature is therefore in no way due to exhaustion. The temperature of the muscle may be raised even up to  $45^{\circ}$ – $47^{\circ}$  for short periods, and the rhythm still returns on lowering the temperature, that is, excitability returns sufficiently for the muscles to be able to respond to the nervous impulses. But if the heart is kept at that high temperature till the heat rigor appears, the cooling no longer restores the rhythm.

When these experiments are made on the preparation represented in Diagram I, Fig. 1, we have no means of knowing whether the ganglion placed in the second cylinder remains active or not at temperatures above  $32^{\circ}$  C., for the only muscular portion in connection with the ganglion has been rendered unable to respond; but when a preparation like Diagram II is made use of, the continued activity of the ganglion is made evident by the continued rhythm by the posterior end of the heart. The failure of the rhythm of the anterior end of the heart at this higher temperature is therefore not due to failure of the ganglion, nor could it be, as the temperature of the ganglion remains unaltered. We shall see later that it is not due to loss of conductivity of the nerves. The seat of paralysis must therefore be the motor nerve endings or the muscle itself. The fact that the portion of the heart in state of heat paralysis will respond to direct stimulation might suggest that the seat of paralysis is in the motor nerve endings; but it is also conceivable that the heart muscle may be able to respond to strong artificial stimulation and at the same time have lost so much of its irritability and contractility that it can no longer respond to the rhythmical impulses from the ganglion. The standstill cannot be due to stimulation of inhibitory nerve endings, as suggested by Cyon for the frog, for the simple reason that there are no such nerve endings in the heart muscle of *Limulus*. The heat standstill at  $28^{\circ}$  or  $32^{\circ}$  C. is not due to exhaustion, because, if the rise in temperature to a point beyond this critical limit is sudden, the contractions cease within five to six seconds.

Temperatures lower than  $10^{\circ}$  act very much like temperatures above  $14^{\circ}$  or  $15^{\circ}$ . The strength of the contractions is gradually diminished till the beats cease entirely at  $0^{\circ}$  or  $-1^{\circ}$  C. Preparations in poor condition may cease to beat with the muscle at the temperature  $+2^{\circ}$  or  $+3^{\circ}$  C.

When the temperature variations are confined to the ganglion-free anterior end of the heart, that is, to the heart muscle, the rate of the



contractions is never altered by them. As the cause of the rhythm does not reside in the muscular tissue, but the muscle beats in response to the impulses from the ganglion reaching it through the lateral nerves, the only way that temperature variations of this ganglion-free end could affect the rate would be by stimulation of afferent paths of a local reflex mechanism and thus by altering the rhythm of the ganglion. I have shown in a former paper that such a reflex mechanism in all probability exists in the *Limulus* heart,<sup>1</sup>

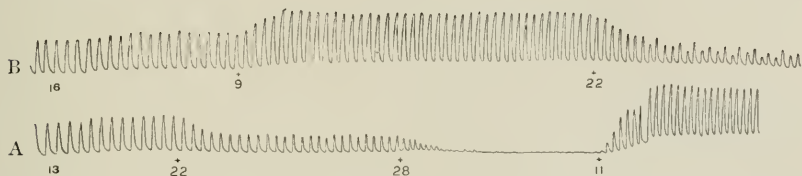


FIGURE 4. — About one-half the original size. *A*, tracing from anterior end of heart ganglion isolated posteriorly (I). Temperature variations of the anterior end, the ganglion remaining at constant temperature; *B*, tracing from anterior end of heart, from which the ganglion had been removed (III). Temperature variations of the anterior end; the temperature of the posterior end remains constant. +, point of sudden change of temperature.

but my present results seem to indicate that it is not stimulated by these temperature variations.

The foregoing points are illustrated in the tracings reproduced in Figs. 4 to 6. Tracing *A* in Fig. 4 from the first two segments of the heart, the nerve cord being isolated posteriorly and left in connection with the anterior end in the manner shown in Diagram I, Fig. 1. The temperature of the anterior end is at 13°. At the point where the temperature is raised to 22° the beats rapidly diminish in strength and remain weaker. When the temperature is raised still higher or to 28°, the beats become almost too weak to record. At this higher temperature the beats are slightly augmented in this tracing. This is due to the action on the ganglion, as it never appeared in preparations in which the ganglion had been removed and placed so that the temperature variations in the first cylinder did not affect it. When the temperature is again lowered to 11°, the strength of the beats returns promptly and exceeds that at 13° C. Tracing *B* is from a preparation with the lateral nerves intact, the nerve cord being removed anteriorly and the muscle in the third segment dissected away. Lowering the temperature of the muscle from 16° to 9° C.

<sup>1</sup> CARLSON: This journal, 1905, xii, p. 471.



strengthens the beats, and raising it to  $22^{\circ}$  brings them down to less than a third. In Fig. 5 the feeble rhythm with the muscle in plasma at  $24^{\circ}$  is entirely stopped when the temperature is raised to  $32^{\circ}$ . The accompanying tonus relaxation is also well marked in this tracing. Lowering the muscle to  $26^{\circ}$  brings back both tonus and rhythmical contractions. The improved rhythm after a period of heat standstill of the muscle is more than usually marked in this record.



FIGURE 5.—Tracing from the ganglion-free anterior end of the heart (III). Posterior end at constant temperature of  $24^{\circ}$  C. Temperature variations on the anterior end. Heat standstill of the muscle.

The temperature of the plasma used to restore the heart is  $26^{\circ}$ , that is, higher and therefore more depressant to the muscle than the original temperature of  $24^{\circ}$ , yet the restored rhythm is more vigorous than the original.

That these alterations in the rhythm of the anterior end of the heart consequent upon the temperature variations are not due to

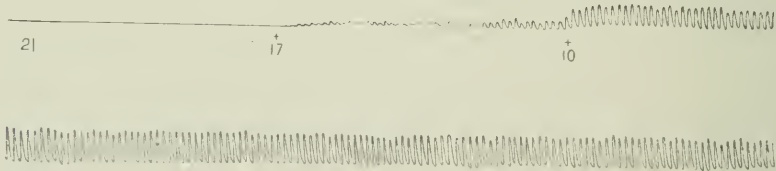


FIGURE 6.—Four-sevenths the original size. Simultaneous tracings from the two ends of the limulus heart prepared as shown in Diagram III, Fig. 1. Lower tracing is from the posterior end, kept at the constant temperature of  $19^{\circ}$  C. Upper tracing is from the anterior end. Influence of temperature variations on the muscle. Absence of reflex effects on ganglion.

alterations in the rhythm of the ganglion is demonstrated by taking simultaneous records from the two ends of the heart. One of these double records is reproduced in Fig. 6. The lower record is from the posterior end of the heart containing the ganglion. The temperature of this portion remains constant at  $19^{\circ}$  C. The upper record is from the anterior end connected only by the lateral nerves, as shown in Fig. 1, Diagram III. At the temperature of  $21^{\circ}$  C. the rhythm of this end is reduced almost to invisibility. At  $17^{\circ}$  C. the

beats become stronger, and still more so at  $10^{\circ}$  C. All this while the rhythm of the ganglion as indicated by the contractions of the posterior end of the heart continues unaltered.

Tracings showing the influence of the lower temperatures on the muscle are not reproduced, as they exhibit nothing new or of particular interest. At temperatures below  $10^{\circ}$  the muscle contracts with gradually lessened strength till it ceases entirely at  $0^{\circ}$  or  $-1^{\circ}$  C.

### III. THE INFLUENCE OF TEMPERATURE ON THE HEART GANGLION.

In the experiments on the influence of temperature on the heart ganglion all three preparations represented in Fig. 1 were used. The influence on the ganglion was measured by the rhythm of the anterior end of the heart, this end being kept at a constant temperature. Variations in temperature influence both rate and strength of the nervous discharges from the ganglion. The influence on these two factors of the rhythm is not necessarily parallel and may indeed appear to be in opposite directions; that is, certain degrees of temperature that diminish the rate may on first sight appear to increase the intensity of the nervous impulses. Certain degrees of temperature augment both the rate and the intensity of the nervous discharges, or the rate may be augmented while the intensity as measured by the muscular response appears to be diminished.

When both the rate and the strength of the beats of the isolated anterior end are augmented by raising or lowering the temperature of the ganglion, it is evident that these changes are due to changes in the ganglion itself. But in case the rhythm of the anterior end is rendered slower and at the same time stronger, or quickened and at the same time rendered more feeble, it is certain that the muscle of the reacting end is a factor that must be considered. Suppose the rate of the nervous discharges from the ganglion is augmented, the intensity remaining the same or being only slightly augmented, the probability is that the beats of the reacting end of the heart will be weakened, because the heart muscle is given less time to recover after each beat. The diminished excitability and contractility of the heart muscle, owing to this shortening of the diastole, may thus obscure or entirely overcome any augmentation in the intensity of the nervous discharges, unless this increase in the intensity is very great. Similarly, in case the rhythm of the reacting end is made slower and stronger, the stronger beats are not

necessarily an indication of stronger nervous impulses from the ganglion, for the slower rhythm of the ganglion gives the heart muscle longer time to recover between the beats. The increase in the excitability and contractility of the heart muscle consequent upon the slower rhythm may thus actually cause stronger beats even in case the nervous discharges from the ganglion are of the same intensity or weaker. Thus the use of the muscle as an indicator of the activity of the ganglion under different conditions of temperature complicates matters so far as regards the intensity of the nervous discharges. This phase of the activity of the ganglion can only be studied accurately by recording the action current of the nerves leading from the ganglion to the muscle.

Although the changes in the intensity of the nervous discharges are thus obscured by synchronous changes in the rate, the tracings indicate that the influence of temperature on rate usually runs parallel with that on the intensity of the nervous discharges. Lowering the temperature diminishes the rate and intensity of the nervous discharges from the ganglion, and raising the temperature augments both. A sudden cooling of the ganglion from  $20^{\circ}$  to  $30^{\circ}$  C. down to  $0^{\circ}$  may in rare cases give a brief augmentation both in rate and strength of the rhythm before the depression of the rhythm sets in. A similar primary augmentation is sometimes obtained in the frog's heart on sudden cooling to  $0^{\circ}$  C. (Cyon, Aristow, Stewart).<sup>1</sup> There is thus a direct relation between the degree of temperature and the rate of automatism of the ganglion. This does not imply that at  $20^{\circ}$  C. the rhythm of the ganglion is four times as rapid as at  $5^{\circ}$  C. or twice as rapid as at  $10^{\circ}$  C., but that the rapidity of the rhythm is invariably augmented by any rise of the temperature between the physiological limits for the ganglion. The physiological limits of the ganglion range from  $0^{\circ}$  or  $-1^{\circ}$  to  $42^{\circ}$  C. The upper physiological limit for the ganglion is thus about  $10^{\circ}$  higher than that for the heart muscle. At  $42^{\circ}$  the rhythm of the ganglion ceases, the muscle of the recording end becomes perfectly quiescent and relaxed in tonus. Any further rise in the temperature of the ganglion produces no effect on the muscle. But the ganglion is not killed by this temperature, for lowering it to  $35^{\circ}$  or  $40^{\circ}$  restores the rhythm promptly. The ganglion may be heated to  $47^{\circ}$  (in one case to  $50^{\circ}$ ) for a short time, and the rhythm will return on lowering the temperature. A temperature of  $47^{\circ}$  will kill the ganglion if

<sup>1</sup> This has recently been contradicted by SNYDER: *Loc. cit.*

maintained from five to ten minutes. A temperature of 50° to 55° C. kills the ganglion in a minute or two.

The augmented rhythm of the ganglion following the rise in temperature is accompanied by what appears to be a tonus contraction in the recording part of the heart muscle. This tonus contraction is more marked the greater and more sudden the temperature change on the ganglion. But this is true only within limits. Thus a change of temperature from 20° to 30° C. usually produces greater tonus contraction than an equally rapid change from 30° to 40° C.

These points are illustrated by the typical tracings in Figs. 7 to 10. The tracing in Fig. 7 is from the anterior end of a heart prepared as shown in Fig. 1, Diagram I. The anterior recording end remains at 15° C. At the beginning of the part of the experiment shown by this record the temperature of the ganglion was 22° C. The rise to 27° doubles the rate of the rhythm. The beats are at the same time weaker and the tonus greatly increased. All these effects are augmented by the further rise to 33°, the beats fusing to an incomplete tetanus. But on the further increase to 39° C. the tonus relaxes, although the rhythm becomes even more rapid. These rapid beats are, however, so weak that they can hardly be distinguished except by the aid of a lens. At 41° C. the activity of the ganglion is suspended, the rhythm of the muscle ceasing and the tonus of the muscle relaxing even further. Severance of the nerve cord near the reacting muscular end would have produced the same results. The rhythm of the ganglion is therefore entirely suspended, or it is a case of failure of the nerves to conduct the impulses to the muscle. But we shall see presently that the nerves retain their conductivity up to 42° or 43° C. It is therefore useless to discuss the

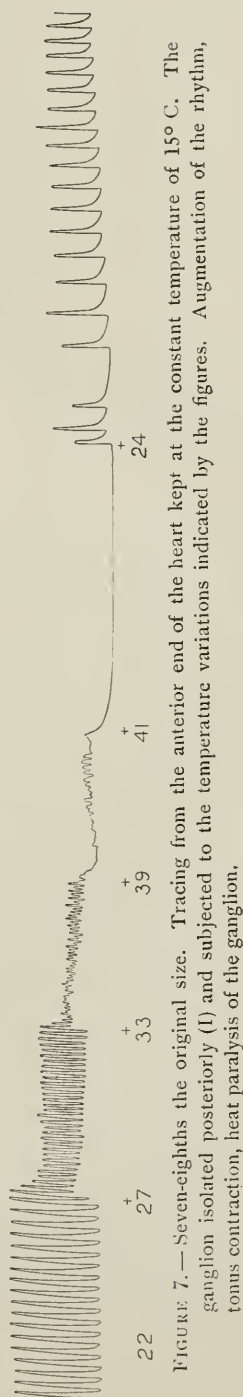


FIGURE 7.— Seven-eighths the original size. Tracing from the anterior end of the heart kept at the constant temperature of 15° C. The ganglion isolated posteriorly (I) and subjected to the temperature variations indicated by the figures. Augmentation of the rhythm, tonus contraction, heat paralysis of the ganglion.

possibility of the ganglion continuing in activity under these conditions, for we have at present no means of proving or disproving it. Lowering the ganglion to  $24^{\circ}$  C. promptly restores its activity. But the rhythm is at first much slower than the original. This is typical. The temperature indicated on the tracing is that of the plasma poured into the cylinder *B* at that point, the ganglion having at that time the temperature of  $41^{\circ}$  C. The equalization between the ganglion and that of the plasma does not take place at once, hence we have a reversal of the principle stated on the previous page. Here the rate

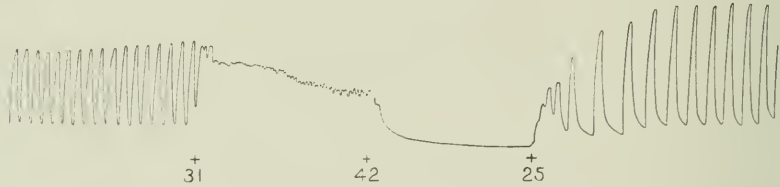


FIGURE 8.— Tracing from anterior end of the *Limulus* heart kept at the constant temperature of  $13^{\circ}$  C. (Ganglion isolated posteriorly (I) and subjected to the temperature variations indicated by the figures. Incomplete tetanus of the muscle, heat paralysis of the ganglion.

is actually augmented while the temperature of the ganglion is falling. This is always true of the recovery of the ganglion after heat standstill.

I have called the rise of the base line of the contractions at  $27^{\circ}$  and  $23^{\circ}$  a tonus contraction. It may also be explained simply as incomplete diastole, the rhythm being so rapid that the muscle does not get time to relax to its normal extent. But the behavior of the muscle at  $39^{\circ}$  appears to me to contradict this explanation, for here the rapidity of the rhythm is actually increased, while the lever nevertheless makes a rapid descent.

When the temperature of the ganglion is suddenly raised from  $20^{\circ}$  to  $30^{\circ}$  or  $35^{\circ}$  C., the anterior end of the heart is often thrown into a state of contraction resembling incomplete tetanus. A tracing showing this reaction is reproduced in Fig. 8. In some preparations this tetanus curve would rise above the level of the normal systole, thus giving a closer resemblance to true tetanus. In Fig. 8 it falls slightly below it.

The experiments on the heart preparation III in which tracings were taken simultaneously from the anterior and posterior ends of the heart demonstrates conclusively the continued activity of the



ganglion at temperatures too high for the muscle to respond to the nervous impulses. They also show that lowering the temperature of the ganglion diminishes not only the rate but also the intensity of the nervous discharges. Two typical records of this series of experiments are reproduced in Figs. 9. and 10. In Fig. 9 the temperature of the two ends of the heart is the same ( $24^{\circ}$  C.) at the beginning of the experiment. The preparation is one in which the ganglion had been removed from the anterior end. The temperature of the

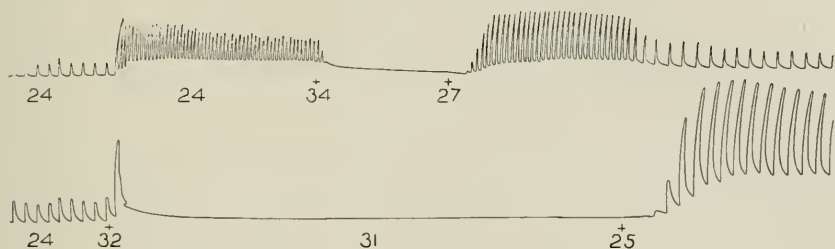


FIGURE 9. — One-half the original size. Simultaneous tracings from the two ends of the heart, prepared as in Diagram III, Fig. 1, to show the persistence of the activity of the ganglion after the heat standstill of the muscle. Upper tracing is from the ganglion-free anterior end, the lower is from the posterior end. The + denotes the point of sudden change of the temperature. For further explanation see text.

posterior end and of the ganglion is then suddenly raised to  $32^{\circ}$  C. This produces a greatly strengthened beat of this end, followed by quiescence in diastole and slight tonus relaxation till the temperature is again lowered to  $25^{\circ}$  C. It is thus evident that the sudden warming of the ganglion with the muscle beyond the point of  $32^{\circ}$  C. produces nearly the same results as the same warming of the heart muscle separated from the ganglion. This is necessarily the case, for whatever the effect may be on the ganglion, the muscle cannot show it. The only difference between heating the muscle by itself and the muscle together with the ganglion is the preliminary strong beat shown on the tracing at  $32^{\circ}$  C. If the rise of the temperature is less sudden than was the case in this experiment, there is always a brief preliminary augmentation before the diastolic quiescence sets in. This never occurs when the muscle itself is suddenly heated. The preliminary augmentation in case of warming of the muscle and the ganglion at the same time must therefore be due to the fact that the ganglion is more quickly stimulated by this rising temperature than the muscle is depressed by it, so that the muscle is able to register the beginning of the augmentation of the ganglionic rhythm.



That such a stimulation of the ganglion actually takes place is shown by the rhythm of the anterior end of the heart in the upper record. In this particular experiment both the rate and the intensity of the nervous discharges must be augmented, for the anterior end beats more strongly and rapidly under the same conditions of temperature and environment. The strength of the beats of this end diminishes gradually, and the beats cease entirely if this temperature of the ganglion is maintained for ten to twenty minutes. This rhythm of the anterior end may be stopped by raising the temperature above the critical point for the muscle. Thus the muscle of both ends of the heart is absolutely quiescent, while the ganglion continues in rapid and vigorous rhythm, as shown by the fact that the rhythm of the anterior end of the heart returns as soon as the temperature of that end is lowered. Lowering the temperature of the posterior ends below the critical point for the muscle restores the rhythm of this end, while the rhythm of the anterior end is greatly slowed and enfeebled. This is invariably the rule. When the temperature of the anterior end is near that of the critical point for the muscle, the lowering of the temperature of the ganglion may stop the rhythm of this end. The return of this slow and vigorous rhythm of the posterior end goes hand in hand with almost complete disappearance of the rhythm of the anterior end. This is easily understood when the heart mechanism is taken into account. We have seen that lowering the temperature of the ganglion always lowers the rate of the rhythm. It will be noted in Figs. 9 and 10 that the beats of the two ends of the heart after restoration of the rhythm of the posterior ends are synchronous. The slowing of the anterior end is therefore nothing but the slowing of the ganglionic rhythm due to the drop in temperature from  $31^{\circ}$  to  $25^{\circ}$  C. But how are we to account for the diminution in the strength of the beats in the anterior end while the beats of the posterior end are augmented? The diminution in the height of contraction of the anterior end can be due to nothing else than a diminution in the intensity of the nervous impulses reaching it, for all other conditions on which the rhythm depends are constant. But it is not necessary to conclude that there is some kind of an antagonistic mechanism in the nerve cord acting so that the nervous discharges reaching the anterior end of the heart are being weakened while those of the posterior end are strengthened, for we have seen that the heart muscle kept for some minutes in quiescence by a temperature slightly above the critical point gives a very vigorous rhythm

for a while on lowering the temperature, the activity of the ganglion on which the rhythm depends remaining the same (Figs. 4 and 5). The increased excitability of the muscle in the posterior end thus more than compensates for the diminution in the intensity of the nervous discharges, so that the beats are stronger although the nervous discharges are actually weaker. It is of interest to note in Fig. 9 that on lowering the temperature of the posterior end the rhythm of the anterior end begins to weaken before the posterior

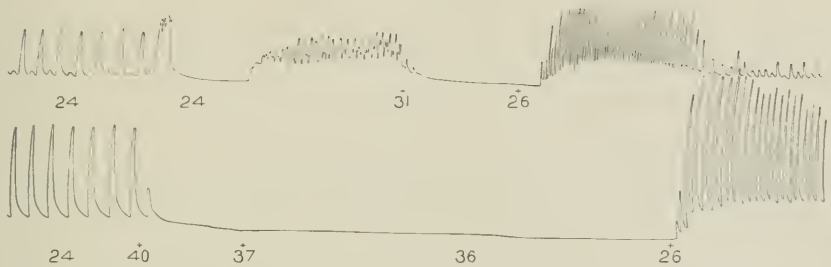


FIGURE 10. — One-half the original size. Simultaneous tracings from the two ends of the heart (III). The figures indicate the temperature variations of either end. Showing heat paralysis and restoration of muscle and ganglion and diminution in the intensity of the nervous discharges from the ganglion on lowering the temperature.

end of the heart shows any sign of activity. This phenomenon is of frequent occurrence. It is evidently another instance of the temperature changes acting more rapidly on the ganglion than on the muscle, the ganglionic rhythm being toned down by the lower temperature before the muscle has recovered sufficiently to respond.

If the temperature of the posterior end is suddenly raised to the critical point of the ganglion, the rhythm of that end of the heart may cease in diastole without any initial augmentation (Fig. 10). This is not due to an instantaneous cessation of the activity of the ganglion, for the anterior end of the heart always shows the characteristic augmentation and tonus contraction for a few seconds. It must be due to the very rapid loss of excitability of the muscle on being suddenly surrounded by plasma at a temperature of  $10^{\circ}$  C. above the critical point. It will be noted in Fig. 10 that the anterior end gives two beats to every one beat of the posterior end. This form of incoordination and also the reverse were met with occasionally. In Fig. 10 the irregularity is seen to persist in the rhythm following the lowering of the temperature of the posterior end to  $37^{\circ}$  C., but has

disappeared after the anterior end has been brought to a heat standstill and the rhythm again restored by cooling.

The lowering of the temperature of the ganglion from  $20^{\circ}$  C. to  $10^{\circ}$  or  $6^{\circ}$  C. diminishes the rate and the strength of the ganglionic rhythm in the same manner as the similar lowering from  $35^{\circ}$  or  $40^{\circ}$  to  $20^{\circ}$  C. In some preparations a slight initial augmentation of the strength of the nervous discharges appears on suddenly lowering the temperature to  $10^{\circ}$  or  $6^{\circ}$  C. When the tracing from both ends of the heart shows

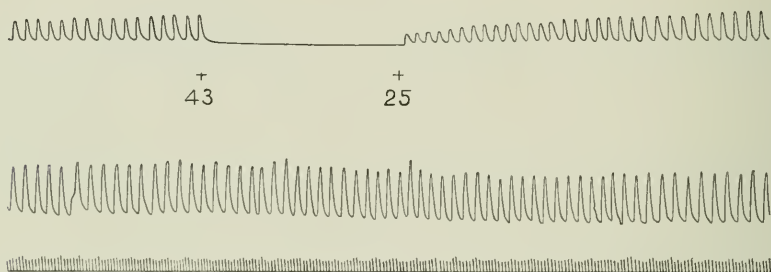


FIGURE 11. — Simultaneous tracings from the two ends of the heart, prepared as in Diagram III, Fig. 1. The two ends of the heart at constant temperature. Local variation of the temperature of the lateral nerves in the third segment, as shown by the figures. Showing heat paralysis of the nerves. Upper record is from anterior, lower record from posterior, end of heart.

an initial augmentation of the strength of the beats before any slowing of the rate has set in, the augmentation in case of the anterior end can hardly be accounted for on any other ground than that the nervous impulses have been slightly strengthened. The augmentation of the posterior end can be accounted for by the increased excitability of the muscle, as  $10^{\circ}$  C. is much nearer the optimum for the muscle than is  $20^{\circ}$  or  $24^{\circ}$  C. A temperature of  $6^{\circ}$  C. is even more favorable for the muscle than that of  $20^{\circ}$  or  $25^{\circ}$  C. The augmentation of the rhythm of the anterior end simultaneous with the slowing of the rate may be entirely accounted for by the slowing of the rate, the muscle having longer diastole in which to recuperate. In by far the greater number of experiments the lowering of the temperature of the ganglion to  $10^{\circ}$  or  $6^{\circ}$  C. did not give any initial augmentation of the strength of the beat of the anterior end, but a speedy decrease, sometimes amounting to complete cessation. The absence or presence of stronger beats in the posterior end evidently depends on the balance between the increased excitability of the muscles and the

diminished strength of the nervous discharges on lowering the temperature of both tissues. When the temperature of both ends of the heart is at  $24^{\circ}$  or  $20^{\circ}$  and the posterior end of the ganglion is suddenly cooled to  $10^{\circ}$  or  $6^{\circ}$ , the rhythm of the anterior end usually ceases completely till the temperature of this end is lowered to the optimum of the muscle.

The activity of a ganglion when in poor condition is stopped in a few seconds by a temperature of  $4^{\circ}$  to  $6^{\circ}$  C. Nerve cords in good condition will maintain a fairly strong but slow rhythm at that temperature for a considerable period. A few ganglia continued in activity for three hours at a temperature of  $4^{\circ}$  C.; towards the end, however, the rhythm was as slow as 1 to 2 beats per minute. At a temperature of  $5^{\circ}$  to  $6^{\circ}$  C. the ganglion may continue in activity for seventy-two hours. The ganglion of very strong and vigorous hearts will continue in activity for some minutes at  $-1^{\circ}$  C. A lower temperature stops it almost at once. These low temperatures do not appear to injure the nerve cord, as the normal rhythm appears on raising the temperature. The lower limit for the ganglion is therefore the same as for the muscle, the exact temperature at which the rhythm ceases depending on the length of time the tissues are subjected to it, both the ganglion and the muscle being able to continue in activity for some time at  $-1^{\circ}$  C. The lowering of the temperature of the posterior end of the heart may produce in addition to the weaker rhythm a peculiar rhythmical variation in the strength of the beats, or a grouping of the beats. A similar phenomenon was sometimes observed after stimulating the ganglion with interrupted current.<sup>1</sup> It is probably of the same character as the Traube-Hering waves in the mammalian blood vessels, that is, a rhythmical tonus variation. The question of the tonus of the *Limulus* heart will be considered in detail in a later paper.

#### IV. THE INFLUENCE OF TEMPERATURE ON THE INTRA-CARDIAC MOTOR NERVES.

For the experiments on the effects of high and low temperature on the conductivity of the intrinsic nerve plexus in the heart, the heart was always prepared as shown in Diagram III, Fig. 1, the lateral nerves only being left in connection with the anterior end of the heart. If the nerve cord is warmed or cooled locally in the region

<sup>1</sup> CARLSON: This journal, 1905, xiii, p. 217.

isolated, that is, in the third segment, variations in the rate of the rhythm are always produced in much the same manner as when the temperature of the whole posterior end of the nerve cord is involved. The nerve cord must therefore be excluded in experiments on conductivity of the nerves at different temperatures. When care is taken that the posterior end of the heart is well isolated from the cylinder containing the loop of the lateral nerves, local variations of the temperature of this loop do not alter the rate of contraction of either end of the heart. The only effects are variations in the strength of the beats of the anterior end. In some preparations an acceleration of the rhythm was obtained when the rise of temperature of the loop of the nerves was great and sudden. Whether this augmentation was due to purely physical conduction of the heat to the ganglion or to stimulation of afferent nerve fibres leading to the ganglion, I cannot tell at present. The fact remains, however, that the temperature of the lateral nerves may in most preparations be raised or lowered beyond the physiological limits without altering the rapidity of the rhythm. These experiments brought out the interesting fact that the nerve fibres are on the whole less affected by variations in temperature than is the heart muscle or the ganglion. The upper physiological limit for the nerve is slightly above that for the ganglion, or about  $42^{\circ}$  to  $43^{\circ}$  C.

The ganglion usually ceases at  $41^{\circ}$  C. The lowering of the temperature of the nerves below  $40^{\circ}$  C. quickly restores the conductivity. The nerves, like the ganglion and the muscle, may be heated up to  $47^{\circ}$  C. for a brief period without being killed. The warming of the nerves above the physiological limit of  $43^{\circ}$  C. produces no effect whatever on the rest of the heart. Whatever changes this heating produces in the nerve, it is not in the line of stimulation.

At the lower temperature the nerves are again more resistant than the muscle, the nerves conducting for some time at  $-2^{\circ}$  C. In the tracing reproduced in Fig. 12 it will be seen that the nerves conduct just as well at the temperature of  $6^{\circ}$  C. as at  $18^{\circ}$  C. The lowering from  $18^{\circ}$  to  $0^{\circ}$  reduces the conductivity of the nerves by one-half in this particular case. Fig. 11 gives a typical tracing showing the heat block of the nerve. The lower record is that of the posterior, the upper record that of the anterior, end of the heart. When the lateral nerves are raised to the temperature of  $42^{\circ}$  C., the beats of the anterior end cease at once, that of the posterior end continuing unchanged.



V. THEORETICAL CONSIDERATIONS.

The influence of temperature variations on the heart of *Limulus* is therefore very complicated. No definite knowledge of these complex influences could have been obtained by following the methods so far used in the investigation of that problem in the vertebrate heart, namely, subjecting the whole heart to the temperature changes.

The persistence of the ganglionic rhythm after the muscle has ceased to respond to the nervous impulses is, it appears to me, the most interesting point brought out by this inquiry, for if a similar condition of things obtains in the heart of cold-blooded vertebrates we should thus have a method of demonstrating the nervous origin of the heart rhythm of these animals. How far can the influence of the temperature variations on the vertebrate heart be interpreted on the basis of the results in *Limulus*? A striking similarity is the fact that local heating of the ventricular end of the heart does not alter the rate of the beats, just as is the case in the *Limulus* when only the muscle and the nerves are heated. Heating the venous end of the vertebrate heart and heating the ganglion in the heart of *Limulus* produce the same augmentation.<sup>1</sup> The venous end of the vertebrate heart is the part most abundantly supplied with ganglion cells. Further analysis between *Limulus* and mammals seems to break down, for, according to Langendorff, there is no heat standstill in diastole in the mammals short of the temperature that produces heat-rigor. But, as has been pointed out, Langendorff's results are modified to some extent by the results of Hertizka, the heat paralysis being obtained in hearts fed with Locke's solution. It is singular that the veins of the bat should, in respect to the heat standstill, behave like the heart of the frog

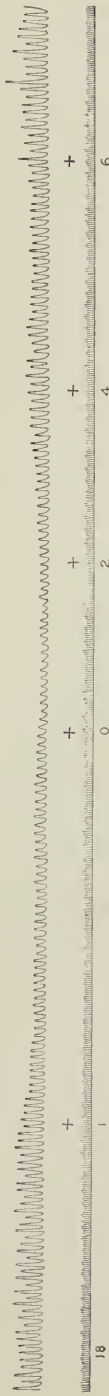


FIGURE 12.—About three-fifths the original size. Tracing from anterior end of *Limulus* heart, prepared as in Diagram III, Fig. 1. Anterior end at constant temperature of 13° C. Local variations of the temperature of the lateral nerves in the third segment, as indicated by the figures. Showing relation of temperature to conductivity in the intrinsic heart nerves.

<sup>1</sup> ADAM: *Centralblatt für Physiologie*, 1905, xix, 39.



and the invertebrates, while the heart of mammals does not. In Langendorff's experiments the whole heart was subjected to the temperature variations. It is possible that the heat standstill in diastole might be demonstrated in the mammalian heart under as nearly a normal condition as the conditions of the experiments would allow by working with an apex preparation of Porter.<sup>1</sup>

In cold-blooded vertebrates and in *Limulus* the similarity of the reaction of the heart at high temperatures is so striking that one is forced to consider the probability of identical mechanisms. Aristow thought that the heat standstill of the frog's heart was due to paralysis of the motor ganglion. Stewart concluded it was due to paralysis of the automatism of the muscle. In *Limulus* we have both these factors; but the standstill due to the warming of the muscle in *Limulus* is not a paralysis of muscular automatism, but a paralysis of the motor nerve endings or diminished excitability of the muscle itself. We have pointed out that this must also be the case in the heat standstill of the frog's ventricle and auricles, when left in connection with the sinus. Whether or not we have in the frog's heart a paralysis of motor ganglia at temperatures different from that of the standstill of the muscle cannot be determined except by further experiments. The fact that the sinus and the auricles of the frog require a higher temperature than the ventricle to cause the standstill may suggest such a condition. On the other hand, Stewart found that the rhythm of electrical tension in the frog's ventricle ceased at the moment visible contractions ceased.<sup>2</sup> This would not be the case if the ganglionic rhythm still persisted. Stewart's experiments were, however, not directed towards this point particularly. Waller and Reid<sup>3</sup> found that the dog's ventricle would under some conditions exhibit the usual rhythm of variations in the electrical tensions even when the ventricle remained perfectly quiescent in diastole. It is, of course, possible that the muscle and the nervous tissues in the vertebrate heart do not exhibit this difference of resistance to high temperature even if the intrinsic nervous tissue bears the same relation to the heart rhythm as in *Limulus*. The spinal cord of the frog is paralyzed by high temperature before the heart or skeletal muscles. It would be of interest to determine whether this is not also the case in *Limulus*.

<sup>1</sup> PORTER: *Journal of experimental medicine*, 1897, ii, p. 391.

<sup>2</sup> STEWART: *Loc. cit.*

<sup>3</sup> WALLER and REID: *Philosophical transactions*, 1888, clxxviii, B, p. 215.

The cause of the paralysis of the muscle and the ganglion at high temperatures considerably below that producing heat rigor and death of these tissues is not clear. That it is not due to a heat coagulation to some of the tissue proteids, a coagulation reversible on lowering the temperature, seems to be shown by the following facts. In the first place, the heat paralysis of the muscle is synchronous with the greatest degree of relaxation of the muscle. Heat coagulation would in all probability produce shortening or contraction of the muscle fibres. Secondly, the point at which the heat paralysis of the muscle and the ganglion sets in is not absolute, but depends on the "vital" condition of the preparations. On the theory that the heat paralysis is due to a reversible heat coagulation, one is therefore forced to the further assumption that the coagulation temperature of this proteid varies with the "vital" condition of the tissue, the proteid coagulating at a lower temperature the "poorer" the condition of the heart. To be sure, such an assumption would seem to be strengthened by the results of Miss Latimer on the heat rigor in fatigued muscles, the heat rigor beginning at a point 8° to 10° C. lower in the fatigued than in the resting muscles.<sup>1</sup>

Winterstein<sup>2</sup> has recently made an extended study of the phenomenon of heat paralysis in the central nervous system of the frog and in various invertebrates. His conclusion is that heat standstill or paralysis is an asphyxiation of the tissues, the high temperature increasing the oxygen need of the tissues beyond the available supply of oxygen, the result being an accumulation of oxidizable substances in the cells which block further activity. Winterstein has determined the actual quantity of oxygen needed by various animals at different temperatures. But his view that the standstill is due to lack of sufficient amount of oxygen does not seem probable, at least for the heart. In the first place, the heart of *Limulus* continues in activity for twelve hours or more in an atmosphere of hydrogen or in sea-water from which all the oxygen has been removed.<sup>3</sup> Its oxygen supply can under these conditions come only from fixed or "stored" oxygen in the heart tissues. In the second place, both the muscle and the ganglion can be brought to a heat standstill with-

<sup>1</sup> LATIMER: This journal, 1898, ii, p. 29.

<sup>2</sup> WINTERSTEIN: *Zeitschrift für allgemeine Physiologie*, 1902, i, p. 129, 1905, v, p. 323.

<sup>3</sup> These and related problems have been investigated by Dr. Newman. The results will soon be reported.

out any preliminary stimulation by a sudden rise of temperature to a few degrees above the critical point for these respective tissues. This takes place in sea-water saturated in oxygen as well as in plasma in which the hæmocyanin is more than normally charged with oxygen. It seems, therefore, very unlikely that the stored oxygen of the tissues, which can maintain the rhythm of the heart for many hours in addition to the oxygen of the plasma or sea-water would be used up in two or three seconds by the muscle not making a single contraction and the ganglion making at the most one or two discharges. It would be different if the paralysis were always preceded by augmentation, as is the case when the rise of temperature is gradual.

The state of excitation of the spinal cord of the frog preliminary to heat paralysis, cited by Winterstein in support of his theory, depends on the presence of the cerebral hemispheres and the rapidity of the rise of temperature. The decerebrated frogs remain perfectly quiescent when very gradually heated to 34° C., at which point heat paralysis of the cord sets in, while sudden heating produces spasms and strychnia-like convulsions even in the spinal frog. The violent activity and spasms of the intact frog preliminary to the heat paralysis, even on slow warming, are therefore extreme "voluntary" efforts to escape. The spinal cord of the frog may thus be heated to the point of paralysis without producing any increased activity of the motor elements.

The observation of Stewart<sup>1</sup> that the critical temperature for the frog's heart is raised by raising the indo-cardiac pressure can hardly be explained on Winterstein's theory. It is well known that within limits an increased pressure in the heart acts as a stimulus to the rhythm. This is also true for the *Limulus* heart as long as the nerve cord is intact.<sup>2</sup> But such an increase in the pressure may be secured under conditions that exclude any increase in the oxygen supply of the tissues. Hence the stimulating action of tension is not due to favoring the oxygen supply.

The fact of a physiological optimum, and the fact that temperatures between the physiological optimum and the upper physiological limit depress the activity of the tissues, go to show that the coefficient of chemical reaction velocities, such as we know them in the test tube, does not suffice to account for the influence of temperatures on such

<sup>1</sup> STEWART: *Loc. cit.*

<sup>2</sup> CARLSON: This journal, 1905, xii, p. 471.

a complex system of elements and chemical and physical processes as the living cell or aggregate of cells. Temperatures above the optimum probably produce a number of unfavorable conditions, physical as well as chemical, for the activity of the tissues, the critical point of the resistance of the tissues to these unfavorable conditions depending on their condition or state of vigor. This is in keeping with what I have found touching the activity of the heart tissues of *Limulus* under other abnormal or unfavorable conditions, such as abnormal osmotic pressures. A ganglion in "good" condition will continue in activity for sixty minutes or more in a pure sugar solution, while a ganglion in "poor" condition ceases its activity in the same solution within eight or ten minutes.

#### SUMMARY.

1. The influence of temperature variations on the whole heart of *Limulus* is the same as that on the heart of cold-blooded vertebrates. There is the same augmentation of the rhythm by the higher temperatures, the same depression of the rhythm by the lower temperatures, the same heat standstill in diastole at  $32^{\circ}$  to  $42^{\circ}$  C., the same return of the rhythm on lowering the temperature, the same heat rigor at  $45^{\circ}$  to  $55^{\circ}$  C.

2. In the *Limulus* heart the heat standstill in diastole of the entire heart at  $32^{\circ}$  C. is due to paralysis of the motor nerve endings or to lessened excitability of the muscle itself to the extent that it is no longer able to respond to the impulses from the ganglion. During this heat standstill the heart muscle retains its excitability to direct stimulation, just as the heart of the frog. This heat standstill in the *Limulus* heart cannot be due to stimulation of inhibitory nerve endings in the muscular tissue, because there are no inhibitory nerve fibres leading to the muscle either from the intrinsic ganglion or the extrinsic cardiac nerves. The optimum temperature of the heart muscle is  $10^{\circ}$  to  $15^{\circ}$  C. Temperatures above or below this depress excitability and contractility. Variations of the temperature of the muscle alter the strength but not the rate of the beats. Temperature variations of the ganglion alter both the rate and the intensity of the rhythm.

3. The rhythm of the ganglion in the *Limulus* heart continues at temperatures up to  $42^{\circ}$  C., at which point it is entirely suppressed and remains so as long as this temperature is maintained. The

activity of the ganglion stands in such a relation to the temperature that within the physiological limits at the beginning of the variation the higher the temperature the greater the rate and the intensity of the nervous discharges.

4. The sudden rise of the temperature of the ganglion to  $35^{\circ}$  or  $40^{\circ}$  C. produces tonus and incomplete tetanus contractions of the heart muscle. The heat paralysis of the ganglion is accompanied by tonus relaxation of the heart muscle.

The tonus of the heart muscle is thus dependent on the intrinsic nervous plexus in the heart in much the same manner as the tonus of the skeletal muscle is dependent upon the spinal cord. During the heat paralysis of the ganglion it is inexcitable to direct stimulation.

5. The intrinsic motor nerves exhibit greater resistance to temperature variations than does the heart muscle or the ganglion, the upper limit for the nerves being about  $43^{\circ}$  C. This heat paralysis of conductivity is rapidly removed by lowering the temperature.

6. The upper and the lower limits beyond which the heart rhythm ceases are not fixed points, but depend on the condition of the heart. Thus a ganglion in good condition continues in activity between  $-1^{\circ}$  C. and  $42^{\circ}$  C, while a ganglion in poor condition is paralyzed by the temperatures below  $4^{\circ}$  C. and above  $38^{\circ}$  to  $40^{\circ}$  C. respectively. The same is true of the heart muscle. In hearts in poor condition the muscle may cease to respond at temperatures as low as  $25^{\circ}$  to  $26^{\circ}$  C.

7. The heat rigor of the heart following the rise of temperature above that producing paralysis of the muscles ( $32^{\circ}$  C.) and the ganglion ( $42^{\circ}$  C.) is due, not to stimulation of the ganglion or the nerves, but to direct action on the muscle. Raising the temperature of the ganglion and the nerves above their critical point produces no effect on the heart muscle.



## GALVANOTROPISM OF VOLVOX.

BY OLIVER P. TERRY.

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A. W. GREELEY,<sup>1</sup> in his paper on the physical structure of the protoplasm of paramœcia, makes the statement that when they are grown in an alkaline medium they go to the kathode, and when grown in an acid medium they go to the anode. In a footnote he states that volvox, when exposed for half an hour or longer to an acid medium, also goes to the anode, the reverse of its usual response.

At the suggestion of Dr. E. P. Lyon, I attempted to corroborate Greeley's statement in regard to volvox, and to study further the effects of chemicals on the galvanotropism of these organisms.

### METHOD.

Paraffin chambers were made by cutting out a channel in paraffin previously melted on to an ordinary microscope slide. Three forms of electrodes were used as follows:

First, Porter's non-polarizable boot electrode; second, platinum foil; and third, filter paper. In using the latter, the paper was connected to platinum foil through small cells cut in the paraffin at the ends of the main channel. The small cells were filled with sodium chloride solution and the filter paper was saturated with it.

The results with the polarizable platinum foil electrodes did not differ from those where non-polarizable electrodes were used. The former, as they were more easily cleaned, were used in the greater part of the experiments. The paraffin chamber finally used was 18 mm. long, 5.5 mm. wide, and 3.5 mm. deep, having a platinum electrode at each end with an exposed surface of 18 sq. mm. The area of cross section of the chamber was 19.25 sq. mm.

<sup>1</sup> GREELEY: Biological bulletin, 1904, vii, p. 3.



The currents used were taken from two to five Daniell cells, connected in series. One cell was not enough to produce a response. The resistance of the animal chamber was determined by the use of a Wheatstone bridge, with induction coil and telephone, according to the Kohlrausch method. The resistance was found to be about 17,000 ohms. A Daniell cell has a voltage of 1.1 volts. The current through the chamber was found by substituting in the formula,

$$C = \frac{EMF}{R}. \text{ Supposing two Daniell cells were used, substituting}$$

$C = \frac{2.2}{17000}$  or .00013 ampere or .13 milliampere, if five cells were used, the current would be .32 milliampere. Then dividing these figures by the area of cross section (19.25 sq. mm.), we get the strength of current per sq. mm. to vary from .0067 milliampere for two Daniells to .0168 milliampere for five Daniell cells.

Technically the unit of current density is taken as one ampere to the sq. mm., but Hermann and Matthias for physiological purposes advocate a unit one-millionth as great and use  $\delta$  to express this unit. Therefore  $\delta$  indicates one-thousandth milliampere per sq. mm. of cross section. On this basis, then, two Daniells gave a current of 6.7  $\delta$ , and five Daniells gave a current of 16.8  $\delta$ .

The different strengths of current thus passed through the paraffin chamber did not affect the responses of the volvox. The stronger currents merely hastened their action. After repeated trials five cells were always used.

The length of time during which the current was applied was also varied. If left on for a longer time than that necessary to produce the primary response, the results were confusing and of no value. After the first definite orientation the colonies wandered back and forth between the poles, at irregular intervals, for some time. As a rule, most of them stopped at the anode and died there; a few remained at the kathode and lived considerably longer than those at the anode. The primary response with the five cells occurred, in most cases, in less than one minute, but this varied with the solution in which the colonies were.

The concentration of the acid and alkali solutions was about  $\frac{1}{600}$ . This turned litmus in about ten to fifteen minutes. Stronger solutions killed the organisms. In the experiments the acid and alkali were added to pond water and then the organisms placed in the solution. In the work with the salts distilled water was used to

make up  $\frac{m}{2}$  stock solutions, and these were diluted with pond water.

Care had to be taken to have the poles of the paraffin chamber at right angles to the direction of the light, because of the strong, positive heliotropism of the organisms. In the work on the effect of colored light wooden boxes were used with one end covered with red or blue glass. After placing the organisms in these boxes, they were left for varying lengths of time.

## OBSERVATIONS.

The following table on the salt and acid action presents typical results:

Exp. No.	Chemical and concentration.	Length of time in the solution.	Time of day of test.	Response and time required.	Response of control.	Remarks.
2	$\frac{m}{500}$ NaCl	12 hrs.	8.43 A. M.	Anode, 2 min.	Anode	All dead at anode in 1 hr. Platinum electrodes used. Fresh culture collected on the same morning of tests. Platinum electrodes used.
4	$\frac{m}{500}$ NaCl	14 hrs.	10.40 A. M.	Anode, 2 min.	Anode	
5	$\frac{m}{128}$ NaCl	10 min.	11.50 A. M.	Kathode, $1\frac{1}{2}$ min.	Kathode	
6	$\frac{m}{256}$ NaCl	25 min.	12.05 P. M.	Kathode, 3 min.	Kathode	Boot electrodes used.
8	$\frac{m}{64}$ CaCl <sub>2</sub>	10 min.	3.45 P. M.	Kathode, 2 min.	Kathode	
9	$\frac{m}{64}$ CaCl <sub>2</sub>	35 min.	4.10 P. M.	Kathode, 1 min.	Kathode	Colonies all dead 16 hrs. later.
11	$\frac{m}{128}$ CaCl <sub>2</sub>	45 min.	4.05 P. M.	Kathode, $1\frac{1}{2}$ min.	Kathode	
12	$\frac{m}{64}$ MnCl <sub>2</sub>	10 min.	11.08 A. M.	Anode, 1 min.	....	
14	$\frac{m}{256}$ MnCl <sub>2</sub>	25 min.	11.23 A. M.	Not positive	....	
17	$\frac{m}{64}$ Na <sub>2</sub> SO <sub>4</sub>	30 min.	11.40 A. M.	Not positive	$\frac{1}{2}$ Anode $\frac{1}{2}$ Kathode	
20	$\frac{m}{256}$ Na <sub>2</sub> SO <sub>4</sub>	40 min.	11.58 A. M.	$\frac{1}{2}$ Anode $\frac{1}{2}$ Kathode	2 min. } $\frac{1}{2}$ Anode $\frac{1}{2}$ Kathode	
22	Distilled water	30 min.	2.37 P. M.	Not positive	$\frac{1}{2}$ Anode $\frac{1}{2}$ Kathode	
23	$\frac{m}{512}$ HCl	25 min.	2.05 P. M.	Kathode, $\frac{1}{2}$ min.	Kathode	Various strengths of current were tried. Colonies lived one day longer in the acid than in the control.
26	$\frac{m}{512}$ HCl	1 hr.	10.00 A. M.	Kathode, 1 min.	Kathode	
28	$\frac{m}{512}$ HCl	48 hrs.	10.00 A. M.	Kathode, 1 min.	Kathode	
30	$\frac{m}{512}$ HCl	75 hrs.	2.30 P. M.	Kathode, 1 min.	Kathode	
32	$\frac{m}{512}$ HCl	100 hrs.	4.30 P. M.	Kathode, $1\frac{1}{2}$ min.	Kathode	

After these and other experiments, I became convinced that it was impossible to control the galvanotropism of volvox by the use of any salts, acids, or alkalies, and sought for some other possible cause for their migration to the anode, which Greeley attributed to acid. Some facts suggested that light might have an influence on the galvanotropic response.

Volvox is a green organism. Light, carbon dioxide, and certain salts are necessary for the life of an organism containing chlorophyll. The latter is very sensitive to light, and is the agent by which the synthesis of carbohydrates, from carbon dioxide and water, is brought about in a cell containing it. Plants placed in the dark produce organic acids. In the light these may be synthesized to carbohydrates under the influence of chlorophyll.<sup>1</sup> The amount of light must be sufficient for the needs of the plant. It is possible that in the laboratory the organisms died of insufficient nourishment caused by improper food supply and poor light. It was impossible to regulate either so that the organisms would live for more than four days. It was noticed that the organisms lost chlorophyll to a large extent before death.

It was also noticed that in the few of the experiments in which tests were made earlier than eleven A. M. the colonies almost invariably went to the anode. With the idea of investigating the effects of varying amounts of light on the organism, the following experiments were performed:

**Culture V.** — Collected 8 P. M., August 10, 1905.

Divided into two parts. One was kept in diffuse daylight; the other was placed in comparative darkness. 5 P. M., August 11, or twenty-one hours later, the cultures were tested. All the colonies which were placed in the light went to the kathode in half a minute. Those which had been in the dark showed no definite response to the current at first; a few went to the anode and a few went to the kathode in about three-quarters of a minute. In about three minutes the majority had collected in the anodic half of the field, where they remained.

10 A. M., August 12, or thirty-eight hours after collection, the colonies in both cultures, those in the dark and those exposed only to the early morning diffuse light, went to the anode.

2.30 P. M., August 12, or forty-two hours after collection, three-fourths of the colonies of both cultures went to the kathode.

10 A. M., August 13, or sixty-two hours after collection, four-fifths of the colonies of both cultures went to the anode.

<sup>1</sup> PFEFFER: *Physiology of plants* (translated by EWART), 1900, i, p. 327.

11.30 A. M., August 13, or sixty-three hours after collection, all the colonies in the light went to the kathode. Those in the dark still went to the anode.

4.30 P. M., August 13, or sixty-eight hours after collection, results were the same as at 11.30 A. M.

On August 14 results very similar to those given for August 13 were obtained, that is, both went to the anode early in the day; the ones in the light changing their response about eleven o'clock. Those in the dark remained constantly anodic during the day.

**Culture VI.** — Collected 5 A. M., August 15, 1905.

5.30 A. M., August 15, culture was divided into four parts, as follows:

A. For control.

B. Placed in the dark.

C. Was made acid,  $\frac{m}{600}$  HCl.

D. Was made alkaline,  $\frac{m}{600}$  NaOH.

A, C, and D were left standing in diffuse daylight.

For variation, in these experiments, the currents used were taken from two to five Daniell cells. No difference was noticed in the response of the volvox to the different strengths of current, except in the rapidity of their response.

Beginning at 6 A. M., August 15, up to 8.30 P. M., all four cultures were tested at various intervals and all the colonies went to the kathode.

7.30 A. M., August 16, twenty-six hours after collection, the colonies of all cultures went to the kathode, with a very few exceptions.

1.00 P. M., August 16, thirty-two hours after collection, the following responses were obtained:

A. Control, one-quarter went to the anode and three-quarters went to the kathode.

B. Those in the dark went to the anode.

C. Those in acid crowded to the kathode in three-fourths of a minute.

D. Those in alkali stayed in the anodic half of the field. None went to the kathode.

2.30 P. M., August 16, thirty-three hours after collection. Some of culture B. which had been in the dark since the morning of the day before, was placed in strong diffuse daylight. At 4.30 P. M. the latter went to the kathode, those in the dark still went to the anode. Those colonies in the acid and alkali had not changed in reaction to the current.

7.30 A. M., August 17, fifty hours after collection. Beginning of third day in the laboratory:

A. Control. Colonies went to the anode.

B. Colonies, in the dark, went to the anode.

C and D. Colonies, in acid and alkali, all went to the kathode. These were in a stronger light than was A. A part of the control was now placed in the dark and a part in strong diffuse daylight. These were tested at 10.30 A. M., and those colonies in the dark still went to the anode. Those placed in the light went to the kathode.

10.00 A. M., August 18, seventy-seven hours after collection. Colonies C and D went to the anode. For three days they had been subjected only to the daily changes of the light in the laboratory. They were placed then in direct sunlight and tested at 10.30 A. M., and went to the kathode.

Other experiments illustrating the ease of change from anodic to cathodic reaction, under the influence of light, were as follows :

Colonies kept in the dark for two days were used. These went to the anode.

10.30 A. M. Some were removed to diffuse daylight.

11.00 A. M. Went to the kathode. Then they were removed to comparative darkness.

1.45 P. M. Again went to the anode. Now placed in moderately strong sunlight.

2.45 P. M. Went to the kathode. Removed to diffuse light.

3.15 P. M. Went to the anode. Placed again in strong sunlight.

3.45 P. M. Went to the kathode.

It is known that chlorophyll is able to utilize only the rays of the red and yellow part of the spectrum. Dr. Lyon suggested that the effects of red and blue light be tried ; the results were as follows :

2.25 P. M. A culture which had been kept in diffuse light and which was easily changed from anodic to cathodic response by exposure to strong light, was used. Some of the colonies were placed under red glass and some under blue glass in the direct sunlight.

3.05 P. M. Those under blue glass responded strongly. About one-third went to the kathode and two-thirds to the anode. Of those under red glass, all went to the kathode immediately.

4.05 P. M. No change in the reaction of either culture.

Repetition of these experiments using diffuse daylight, instead of direct sunlight, always produced the same result.

To determine whether the change of temperature, produced by placing the cultures in direct sunlight had any effect, the following experiments were performed :



A fresh culture taken from the pond showed a temperature of  $15^{\circ}$  C. The colonies went to the kathode. After standing in the laboratory for one hour, the temperature had risen to  $19.5^{\circ}$  C. with no change in their kathodic response.

The temperature of some of the same culture was lowered to  $10^{\circ}$  C. for ten minutes, with no change of galvanotropism. The temperature of a few colonies was raised to  $30^{\circ}$  C. for ten minutes, with no effect on their galvanotropic response.

Colonies were now taken which had been kept in the dark for two days. These went to the anode. After an exposure of thirty minutes to sunlight they went to the kathode. During their exposure to the sunlight, their temperature was raised from  $23^{\circ}$  C. to  $35^{\circ}$  C. by the heat of the sun. More of the colonies, which had been kept in the dark, were raised from  $23^{\circ}$  C. to  $35^{\circ}$  C. without exposure to light. These were tested, and still went to the anode.

When paramœcia are placed in solutions of various chemicals, a reversal of cilia occurs, and the reversal of galvanotropism, which occurs in such solutions, has been attributed by Statkewitsch<sup>1</sup> to the same reversal of the cilia. During all of the experiments on volvox the organisms were closely watched to determine whether there was any such reversal of cilia as would be indicated by a backward motion. None was observed, the organisms always turning when the direction of their progress changed. The anterior end of volvox can be distinguished by the smaller amount of chlorophyll there than in any other part. Also, where daughter colonies are present they are situated in the posterior two-thirds of the mother colony. Holmes<sup>2</sup> notes that there is no reversal of cilia when the heliotropism of volvox is reversed. It is also interesting that Holmes found that the heliotropism of this organism is reversed by strong light. This may be related to the reversal of galvanotropism by light which I have described.

A few micro-chemical tests were attempted to determine if possible any internal changes in the colonies, but these have proved unsatisfactory so far and will be taken up again at a later time.

<sup>1</sup> STATKEWITSCH: Dissertation, Moscow, 1903; cited by Jennings, *Journal of comparative neurology and psychology*, 1905, xv, p. 528.

<sup>2</sup> HOLMES: *Biological bulletin*, 1903, iv, p. 319.



## THEORETICAL.

Much work has been done upon the galvanotropism of *amœba*, *paramœcium*, etc. Some of these forms, like the two mentioned and *stentor* and *volvox*, are naturally negatively galvanotropic. Others, like *polystoma* and *opalina*, are normally positively galvanotropic.

These forms have been studied repeatedly, but no prior work, so far as known, has been carried on with *volvox*, or other green forms, which shows the relation of light to their galvanotropic response; nor has the galvanotropism of *volvox* been studied after what are apparently degenerative changes have set in.

Plant physiology tells us that organic acids are produced by plants in the dark and are gotten rid of in the light, the latter process being carried on through the influence of the chlorophyll. It is possible, then, that the acids thus produced in the organisms are responsible for my chief result. This is, that *volvox*, after it is kept in the dark a certain length of time (two or three days) goes to the anode, reversing its normal response. Why the colonies collected in the morning and those kept in the dark for less than two days do not go to the anode, might be explained by supposing that not enough acid is produced in this length of time to effect the change in the protoplasm. Against this idea of organic acid action it will be recalled that I was unable to reverse the galvanotropism by the addition of acid to the culture medium. Greeley, however, claimed to get a reversal with acid. Greeley's colonies must have been in a different physiological state than mine, or else he was led into error by using a culture which had been kept too long in the laboratory.

Another possible explanation of my result is that the galvanotropism of *volvox* is intimately related to the synthesis of starch. After an exposure to darkness for some time, the supply of starch would be used up. Then the colonies were anodic. A short exposure to strong light would start the synthesis and possibly would reverse the response to the electric current. Perhaps the using up of the carbon dioxide in synthesis or the production of oxygen is the determining factor in the reversal.

The most that can be stated definitely is that the galvanotropism of *volvox* is intimately related to the activity of chlorophyll. This is indicated by the fact that red light produces the cathodic response, while blue light produces little effect.

SUMMARY.

1. Volvox when in its normal condition goes to the kathode.
2. Cultures of volvox kept for some time in media containing acids, alkalies, and salts, in insufficient quantity to kill, are not affected in their galvanotropic response by such media.
3. Volvox is unaffected in its galvanotropism by moderate changes of temperature.
4. If kept in the dark for two or three days, the response of volvox is changed from kathodic to anodic. This may then be reversed at will by exposure to light.
5. Whether anodic or kathodic, volvox always progresses with the same end (anterior) forward.
6. Blue light has little or no effect.
7. Red light, which has the power to stimulate assimilation, affects the organisms like sunlight.
8. The galvanotropism of volvox depends upon its state of chlorophyll metabolism.

My thanks are due to Dr. E. P. Lyon for many suggestions. I am indebted to the Carnegie Institution for the use of one of its tables in the Physiological Department of the Marine Biological Laboratory at Woods Hole.

# THE LATERAL BLOOD "PRESSURES"<sup>1</sup> AT DIFFERENT POINTS OF THE ARTERIAL TREE.

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## INTRODUCTION.

OUR knowledge of the changes in the arterial "pressures" which occur as one proceeds from the heart towards the periphery, is somewhat limited.<sup>2</sup> The diagrams in our text-books show merely a line falling gradually until a point is reached which corresponds to the region of the arterioles, after which the line falls more abruptly.<sup>3</sup> Such diagrams show, of course, only the variations in the mean pressure, so that for the sake of completeness some authors have amplified them. Thus Fredericq<sup>4</sup> has replaced the single line by a series of pulse waves which decrease in amplitude and finally disappear altogether in the capillaries. On the other hand more recent writers<sup>5</sup> have added to the single line representing the mean pressure two other lines, one above and one below, the former representing the systolic, the latter the diastolic pressure, and all three meeting at a point where the arterial pulse becomes imperceptible. All these diagrams, however, express only qualitative and approximate relations, and therefore leave much to be desired.

Bearing in mind these facts and observing also the growing interest which is being taken in the relation of the systolic to the

<sup>1</sup> In this, as in a previous communication (*Journal of experimental medicine*, 1906, vii, 1), the author has used the term "pressures" to designate the systolic, mean, diastolic, and pulse pressures when considered collectively.

<sup>2</sup> For a summary of the work of the earlier observers, see TIGERSTEDT: *Physiologie des Kreislaufes*, 1893, p. 351. The subject appears, however, not to have attracted more recent investigators.

<sup>3</sup> FOSTER: *A text-book of physiology*, New York, 1896, p. 161; YEO: *A manual of physiology*.

<sup>4</sup> FREDERICQ; *Elements de physiologie*, 3d ed., 1893, p. 112.

<sup>5</sup> HOWELL and BRUSH: A critical note upon the methods of measuring blood-pressure, *Massachusetts Medical Society*, June 12, 1901.

diastolic pressure and the importance<sup>1</sup> which has been recently attributed to the pulse pressure,<sup>2</sup> it has seemed desirable to undertake a systematic study of these pressure variations in the different arteries of the dog under conditions as nearly normal as possible.

#### METHOD.

At first sight the ideal method of determining the pressures in the various parts of the arterial tree would seem to be that of connecting each of the vessels to be examined with a separate recorder, so as to obtain simultaneous records of the pressures occurring in each. There are, however, two insuperable objections to this method. In the first place, it is always to be desired that such instrumental errors as are quite unavoidable should be the same for all the arteries examined, so that the results, if not quite accurate, may be at least comparable; whereas the employment of several recorders each with its own errors would render the process of making trustworthy readings difficult, if not impossible. In the second place, the greater the number of the arteries ligated, the more abnormal do the conditions become under which the circulation is carried on. Since then this "ideal" method is quite impracticable, it was necessary to devise another which would avoid the two sources of error above mentioned.

The writer therefore employed the same recorder in all cases and ligated very few of the larger arteries in any one animal. The use of but a single recorder precluded the possibility of taking simultaneous records. This defect, however, was of no great practical importance, since the apparatus was so arranged that the recorder could be disconnected with one artery and connected with another by the mere turning of a stopcock, so that, in the twinkling of an eye, the record of the carotid, for instance, might be made to succeed that of the femoral and *vice versa*. In avoiding the ligation of more than a few of the large arteries in each animal, a more serious difficulty was encountered, for since under these circumstances several animals had to be used, it became necessary to compare the pressures in one dog

<sup>1</sup> ERLANGER and HOOKER: The Johns Hopkins Hospital reports, 1904, xii, p. 145.

<sup>2</sup> Each ventricular contraction causes a wave of positive pressure (pulse) to pass along the arterial tree towards the periphery. Hence the rhythmic change in the arterial pressure at any given point has been designated the pulse pressure. It is, of course, calculated by subtracting the diastolic pressure at the point in question from the corresponding systolic pressure.

with those in another. If, for instance, in a given dog the pressures in the renal artery were observed, then it was not considered advisable to dissect out and take readings from the subclavian in the same animal. It was therefore necessary to reduce all the pressure values to a common standard, even though the final results should thereby indicate not the pressures found in any one animal, but the relative pressures in an imaginary, average, or "schematic" dog.

Suppose we desire to compare the systolic pressure in the cœliac with the systolic pressure in the renal. We cannot take simultaneous records from these two arteries for various technical reasons. We therefore take two dogs A and B, and determine in A the systolic pressure in the cœliac (CA) and femoral (FA) arteries, and in B the systolic pressure in the renal (RB) and femoral (FB) arteries. Having obtained these four values, we can divide as follows:  $\frac{CA}{FA}$  and  $\frac{RB}{FB}$ . This gives us the values of CA and RB in percentages of the femoral pressures in A and B respectively, and these values are, of course, comparable. This is the simplest method, but, unfortunately, it furnishes data which cannot easily be used for the construction of such diagrams as are presented in this article. Consequently another device had to be employed, which is as follows: Suppose, as before, that CA and FA are known, and suppose also that we know the average systolic pressure (F) in the dog. Then F is, by definition, the pressure in the femoral artery of the average or schematic dog. Our task is to determine the systolic pressure in the cœlic artery (C) of this schematic dog, and this can be done from the following equation,  $\frac{C}{AC} = \frac{F}{AF}$  or  $C = \frac{AC \times F}{AF}$ , which is quickly solved with the aid of logarithms. In like manner, when RB and FB are known,  $R = \frac{RB \times F}{FB}$ . Here F, C, and R are all comparable, and represent the femoral, cœliac, and renal systolic pressures in the schematic animal. Naturally, the diastolic and mean pressures can be determined in the same way.

In selecting a standard artery, *i. e.*, one with which all other arteries could be compared, it was obviously advisable to choose a vessel which would be readily accessible. The femoral was therefore chosen as best meeting this requirement. There are, however, occasions in which the employment of this artery would be quite out of the question. This would be the case when the end pressures in the saphenous or iliac were to be determined. Here it would be necessary, or at least desirable, for the blood flow to be normal in both femoral arteries. At such times, therefore, the end pressure of the left carotid was used as a standard, the method of making the necessary calculations being *mutatis mutandis* carried out as before; only, be it remembered, we have here to deal not with the average of numerous carotid pressures as actually deter-



mined, but with the carotid pressures in the schematic dog, *i. e.*, the average of the actual pressures after these have been reduced to the femoral standard.

It is not to be expected that the blood pressures in such arteries as are difficult of access will be found in an exactly normal condition. The operative procedure is in many cases a disturbing factor which can neither be eliminated nor corrected mathematically. The presence and the magnitude of such errors should, however, be known, and in order to obtain this information each experiment was performed in the following way. The first step consisted in laying bare and cannulating a standard artery. This, since it was either the carotid or femoral, could be reached very quickly, and determinations of the pressures made early in the experiment. The second artery was then sought out, and a reading from this second artery compared with a second reading from the standard artery. If in the meanwhile any considerable change had occurred in the pressures, owing to the operation or the anæsthetic,<sup>1</sup> it could readily be detected by comparing the first and second readings from the standard artery.

**Recording apparatus.**—In the selection of a recording apparatus certain instrumental difficulties were encountered. It is well known that the mercury manometer is worthless for recording the rapid changes of pressure which occur in the circulation. Consequently, when maximum and minimum pressures are to be measured, it is customary to employ one of the two other kinds of manometers, namely, the valved or the elastic manometers. As examples of the first class there are the manometers described by Goltz and Gaule,<sup>2</sup> and by Hürthle;<sup>3</sup> while of the second class may be mentioned the well-known "Hürthle manometer"<sup>4</sup> and the instruments devised by Fick.<sup>5</sup> For reasons which need not be discussed in this article the writer preferred the use of the valved manometer.

In order to facilitate and expedite the recording of the blood pressures, an apparatus of a somewhat complicated character was devised. The arrangement of the more essential parts is given in the accom-

<sup>1</sup> Each animal received from 0.4 to 1.2 gm. morphia hypodermically one hour before the beginning of the experiment. During the experiment ether was given at first with a cone, but later through a tracheal cannula.

<sup>2</sup> GOLTZ and GAULE: *Archiv für die gesammte Physiologie*, 1878, xvii, p. 102.

<sup>3</sup> HÜRTHLE: *Ibid.*, 1888, xliii, p. 426.

<sup>4</sup> HÜRTHLE: *Ibid.*, p. 399.

<sup>5</sup> FICK: *Archiv für Anatomie, Physiologie und wissenschaftliche Medizin*, 1864, p. 583; also *Archiv für die gesammte Physiologie*, 1883, xxx, p. 597.



panying sketch, in which are shown two valves,  $MiV$  and  $MxV$ , one "alternate" stopcock  $B$ , four 3-way stopcocks, and a series of connecting tubes. Of the tubes leading from the system,  $Mi$ ,  $Mx$ , and  $M$  communicate with a mercury manometer;  $A'$ ,  $A''$ , and  $A'''$  with the same pressure bottle containing distilled water;  $O$  with a rubber tube running to waste jar; and  $I$  and  $II$  with the arteries to be examined. The valves were made by tying a piece of gold-beaters' skin over a slit or round hole in a glass tube, and are therefore essentially like those described by Williams.<sup>1</sup>

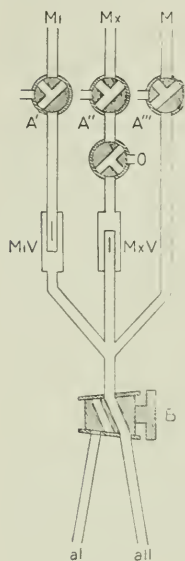


FIGURE 1.

The method of taking the readings can best be explained by describing a typical experiment. Let the problem be to compare the pressures in any two arteries ( $aI$  and  $aII$ ).

*First manœuvre*, to determine the line of zero pressure or base line. Stopcocks arranged as in Fig. 2. Pressure bottle lowered to the level of the arteries to be examined, and the corresponding position of the mercury columns recorded on the kymographion. Should the arteries  $aI$  and  $aII$  be at different levels, it would be necessary to determine the amount of this difference so that the proper correction could be made in the readings subsequently obtained.

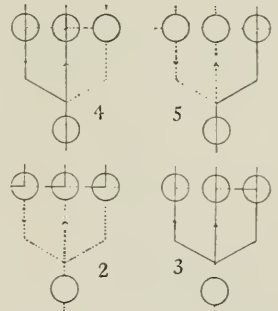
*Second manœuvre*, to test the valves. Stopcocks arranged as in Fig. 3. Pressure bottle raised and lowered alternately. Manometer  $M$  rises and falls with the rising and falling of the bottle. If, on raising the bottle, manometer  $Mi$  does not rise, and if, on lowering it, manometer  $Mx$  does not fall, then the valves are competent. If, on raising the bottle,  $Mx$  rises *pari passu* with  $M$ , and if, on lowering the bottle,  $Mi$  falls *pari passu* with  $M$ , then the valves have no inertia. Of course, as a matter of fact, inertia is never absent, but its amount can be determined by noting, by means of  $M$ , the pressure required to open each of the valves. If the inertia thus determined exceeds 6–8 mm. Hg, then the valve must be discarded. It is easy to show by tapping lightly on the rubber tube connecting  $A'''$  with the pressure bottle, that the valves open much more easily when subjected to an intermittent than to a steady or to a slowly changing pressure. Hence in an actual experiment the error due to inertia is less than that shown by this method of testing; probably it is considerably less, though it is impossible to say just how much.

*Third manœuvre*, to compare the pressures in  $aI$  and  $aII$ . (1) Determination of the maximum and minimum pressures. Arrangement of the stopcocks as in Fig. 4. The maximum and minimum pressures are determined simul-

<sup>1</sup> F. WILLIAMS: Archiv für experimentelle Pathologie und Pharmakologie, 1881, xiii, p. 3.

taneously in one of the arteries. Usually the one which is thought to have the smaller pulse pressure is taken first. Then, by merely turning *B*, corresponding readings from the other artery are obtained. If it be desired to repeat these observations, the pressure can be raised in the minimum manometer by connecting it with the pressure bottle through *A'*, while the maximum manometer can be lowered by being connected with the outflow tube *O*. (2) Determination of the mean pressure. Arrangement of stopcocks as in Fig. 5.

The mean pressure is determined in the two arteries alternately (several readings being made) by suitably manipulating the stopcock *B*. For, as the excursions of the mercury column which correspond to the heart beats are decreased in amplitude by constricting the aperture which connects the manometer with the animal, the position of the mercury column approaches more and more closely the line of mean pressure. It is easy therefore to determine the mean pressure by turning the stopcock *B* until the excursions of the mercury column become almost nil, this being the method described by Setchenow.<sup>1</sup>



FIGURES 2-5. — The solid lines show open connections; the broken lines indicate connections which have been interrupted by turning of the stopcocks.

It may be urged that in an apparatus possessing so many rubber joints much of the pulse wave must be lost. The error due to the yielding of the apparatus is probably not very great, for on measuring the extensibility of the whole system it was found to be only 0.3 c.c. for a rise in pressure amounting to 150 mm. Hg; and since it never happens that all the tubes are in use at the same time, some of them being cut off by the stopcocks, the amount of actual extension during an experiment is probably considerably less, though it is impossible to say just how much.

#### THE ANATOMICAL RELATIONS OF THE ARTERIAL TREE.

The larger branches of the arterial tree in the dog are shown in the accompanying diagram (Fig. 6). From this it is clear that if it be desirable (as it is in the present instance for the sake of convenience) to divide the tree into regions or systems, that manner of division which would accord best with the anatomical relations would be the following: I, aortico-femoral system, II, brachio-cephalic system, and III, left subclavian system.

<sup>1</sup> SETCHENOW: *Zeitschrift für rationelle Medizin*, 1861, xii, p. 334.

## RESULTS.

Since the details of the individual experiments are entirely unimportant, one may pass on at once to a consideration of the results.

**The standard arteries.** — As already stated, the pressures taken as standards for comparison were the end pressures in the left femoral

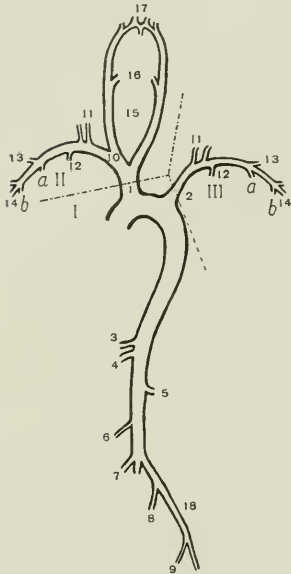


FIGURE 6.—Diagram showing some of the larger branches of the arterial tree in the dog. *I* is the aortico-femoral system; *II*, brachio-cephalic system; *III*, left subclavian system. 1, is the brachio-cephalic artery; 2, left subclavian; 3, coeliac; 4, superior mesenteric; 5, left renal; 6, inferior mesenteric; 7, right iliac; 8, left deep femoral; 9, left saphenous; 10, innominate; 11, vertebral (right and left); 12, internal mammary (right and left); 13, axillary (right and left); 14, brachial (right and left); 15, carotid (right and left); 16, thyroid (right and left); 17, circle of Willis; 18, left femoral. *a* and *b* are branches (schematic) arising just proximal to the axillary and brachial arteries respectively (see page 253). This diagram is given for the purpose of rendering more lucid the presentation and discussion of the results of this investigation.

and left carotid arteries. Consequently it has seemed proper to refer to these arteries first. The femoral standard was obtained by averaging the pressures observed in twenty dogs, while the carotid standard represents the average of the “reduced” pressures obtained in nine animals. These values are shown in the table on page 251.

**The aortico-femoral system.** — The pressures in the aortico-femoral system were determined according to the method above described by inserting cannulas into nine different branches. The actual pressure values were “reduced” in the usual way, and the averages of these “reduced” values, which contain, of course, the gist of the observations, are given in the table on page 252, and are subsequently presented in the form of a chart (Fig. 7).

If one regard the aortico-femoral system as a tube extending from the heart through the femoral artery, into which piezometers (namely,

the branch arteries) have been inserted at various points, then the end pressures determined in these branch arteries would be equal to the lateral pressures in the aortico-femoral tube. If, however, we obtain end pressures from the iliac artery, then one is no longer taking end pressures from a branch, but from the main trunk of the system. The arterial tube can no longer be regarded as extending

	Systolic pressure.	Mean pressure.	Diastolic pressure.	Pulse pressure.	Percentage pulse pressure. <sup>1</sup>
Femoral (left)	188	120	95	93	49
Carotid (left)	162	122	103	49	36

<sup>1</sup> By "percentage pulse pressure" is meant the value obtained by dividing the pulse pressure by the systolic pressure and multiplying by 100. It is the ratio of the pulse pressure to the systolic pressure.

from the heart through the femoral, but from the heart through some branch just proximal to the iliac, such as the other iliac. *Mutatis mutandis* the same is true of the end pressure taken in the femoral, for the end pressures of the femoral and lateral pressures of an arterial tube which extends from the heart through an artery arising from a point just proximal to the femoral, namely, the deep femoral. Consequently it is not permissible to compare end pressures obtained in the iliac and femoral with end pressures in the other arteries of this system. In the chart, therefore, the pressures from the branch arteries are included in the curves composed of solid lines, while the pressures from the main trunk are placed by themselves and joined together by a broken line.

**The brachio-cephalic system.**— Unlike the descending aorta, the brachio-cephalic artery at once breaks up into several branches of nearly equal size, and consequently the brachio-cephalic system can be considerably subdivided. The subdivisions which have been adopted by the author for convenience in chart-making are the aortico-cephalic and the aortico-brachial. These names are self-explaining.

What has already been said with regard to the end pressures in the iliac and femoral arteries applies with equal force to the end pressures in the axillary and brachial, for the axillary and brachial are parts of the main trunk of the aortico-brachial system, just as the iliac and femoral are parts of the main trunk of the aortico-femoral

AORTICO-FEMORAL SYSTEM.						
Name of artery observed.	Systolic pressure.	Mean pressure.	Dias-tolic pressure.	Pulse pressure.	Percent-age pulse pressure.	No. of animals observed.
Brachio-cephalic . . . . .	163	121	103	60	36	3
Subclavian (left) . . . . .	168	123	105	63	37	3
Cœliac . . . . .	171	121	96	75	43	3
Mesenteric (superior) . . .	168	123	95	73	43	1
Renal (left) . . . . .	165	123	103	62	37	3
Mesenteric (inferior) . . .	159	119	95	64	40	2
Iliac (left) . . . . .	183	118	92	91	49	3
Femoral (deep, left) . . .	152	118	102	50	32	3
Saphenous (left) . . . . .	134	118	102	32	23	2
BRACHIO-CEPHALIC SYSTEM.						
Innominate . . . . .	} 160	123	101	59	31	} 2 9
Carotid (left) . . . . .						
Carotid (right) . . . . .	154	119	102	52	34	3
Vertebral (right) . . . . .	} 154	120	104	50	32	} 2 2
Mammary (internal, right)						
Axillary (right) . . . . .	155	117	101	54	35	3
Brachial (right) . . . . .	156	118	101	55	35	3
Thyroid (right) . . . . .	} 140	118	97	43	31	} 3 3
Thyroid (left) . . . . .						
Circle of Willis . . . . .	....	104	....	....	....	2
LEFT SUBCLAVIAN SYSTEM.						
Vertebral (left) . . . . .	163	121	102	61	37	3
Mammary (internal, left) . .	....	....	....	....	....	3
Axillary (left) . . . . .	161	123	109	52	32	3
Brachial (left) . . . . .	160	118	110	50	31	3



system. To make this evident in the chart (Fig. 8), the same device has been adopted as in the case of the iliac and the femoral, namely, the uniting of these pressures with a broken instead of a solid line.

It is unfortunate that readings could not be obtained from such arteries as *a* and *b* (Fig. 6), but it was found that with the valved manometer determinations from such small arteries could not be relied upon.

The results obtained in the case of this system are presented in the above-mentioned table (page 252), and in the form of two charts (Figs. 8 and 9).

**The left subclavian artery.** — What has already been said under the heading "brachio-cephalic system" with regard to the axillary and brachial pressures is equally applicable to this system also. The results have been tabulated and charted as before (table on page 252 and Fig. 10).

Table of average "reduced" end<sup>1</sup> pressures of the various arteries named. The data presented in this table were employed in constructing the following charts (Figs. 7-10).

The chart (Fig. 7) shows that as one proceeds towards the periphery the mean and diastolic pressures remain unchanged. The systolic pressure is found to diminish considerably when the readings are taken in branches of the main trunk. When taken in the main trunk itself (iliac and femoral arteries), the systolic pressures are very high.

The chart (Fig. 8) shows that there is little or no change in the mean and diastolic lateral pressures in the aortico-brachial trunk between the aorta and the origin of the internal mammary, while the systolic pressure is slightly diminished. It may also be seen that the end pressures in the axillary and brachial arteries are about equal to the lateral pressures of the aorta at the origin of the subclavian artery.

The chart (Fig. 9) shows that by the time the thyroids are reached the systolic pressure has become appreciably smaller, also that in the

<sup>1</sup> The pressure designated "circle of Willis" was obtained by inserting a cannula into the distal portion of the common carotid, the external carotid having been ligated. This is therefore the lateral pressure in the circle, while the pressure designated "vertebral," for example, is the end pressure in the vertebral, and so with all the other arteries enumerated in the table. Owing to the small size of the pulse pressure, the systolic and diastolic pressures in the "circle of Willis" could not be obtained by means of the valved manometer.



circle of Willis the lateral mean pressure is low. In the circle the lateral systolic is very greatly diminished, but the pulse pressure is then so small that neither the systolic nor the diastolic pressures could be accurately determined with the valved manometer.

The chart (Fig. 10) shows essentially the same features as Figure 8.

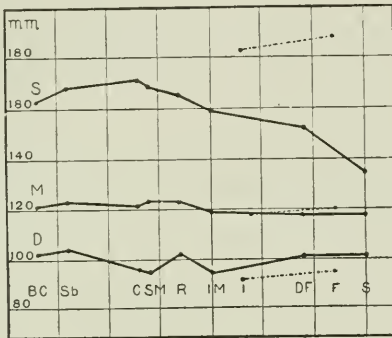


FIGURE 7. — Aortic-femoral system. *S*, *M*, and *D* are the systolic, mean, and diastolic end pressures respectively. The solid lines connect end pressures taken in side branches of the aortic-femoral trunk. Broken lines connect pressures taken in the main trunk of the system. *BC* is the brachio-cephalic artery; *Sb*, left subclavian; *C*, cœliac axis; *SM*, superior mesenteric; *R*, left renal; *IM*, inferior mesenteric; *I*, iliac; *DF*, deep femoral; *F*, femoral; *S*, saphenous.

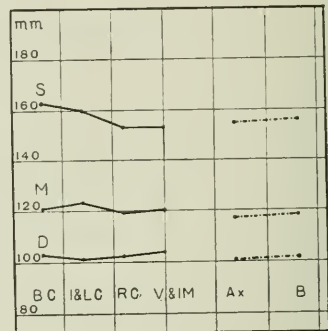


FIGURE 8. — Brachio-cephalic system, aortico-brachial division. *S*, *M*, and *D* as before. Solid and broken lines as before. *BC* is the brachio-cephalic artery; *I*, innominate; *LC*, left carotid; *RC*, right carotid; *V*, right vertebral; *IM*, right internal mammary; *Ax*, right axillary; *B*, right brachial.

#### DISCUSSION AND CONCLUSIONS.

Attention will now be directed to certain facts disclosed by inspection of the foregoing charts as also to certain data not yet presented.

1. The mean pressure is practically constant throughout the large arteries.<sup>1</sup> It is not until such small arteries are reached as those which enter into the formation of the circle of Willis that any conspicuous decrease in this pressure is observed.

2. The diastolic pressure is also constant in the larger arteries<sup>2</sup> with the single exception to be mentioned later.

<sup>1</sup> A fact already noted by POISEUILLE: *Recherches sur la force du cœur aortique*, Thèse, Paris, 1828, p. 31.

<sup>2</sup> As already noted by HÜRTHLE working with his elastic manometer: *Archiv für die gesammte Physiologie*, 1890, xlvii, p. 34.

3. When one considers the variations in the systolic pressure, it becomes absolutely essential to distinguish between the end pressures obtained from the branches of the main arterial trunk and those obtained from the main trunk itself. In the former case the systolic pressure shows a steady and considerable falling off which becomes apparent in end pressures taken in the thyroid arteries, in branches

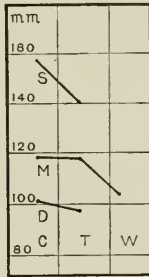


FIGURE 9.—Brachio-cephalic system, aortico-cephalic division. *S*, *M*, and *D* as before. *C* is the carotid arteries; *T*, thyroids; *W*, circle of Willis. The pressure called *W* is the lateral pressure in the circle of Willis, while the other pressures are end pressures in the carotids and thyroids respectively (see page 253, foot-note).

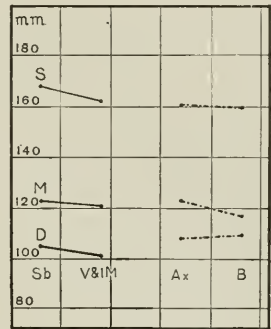


FIGURE 10.—Left subclavian system. *S*, *M*, and *D* as before. Broken and solid lines as before. *Sb* is the subclavian artery; *V*, left vertebral; *Ax*, left axillary; *B*, left brachial.

arising from the axillary,<sup>1</sup> and in branches arising from the lower part of the aortico-femoral trunk.

4. When, however, the systolic end pressure is taken in the main arterial trunk, it is found that this pressure either remains high (axillary and brachial), or may even greatly exceed the corresponding lateral pressures in the aorta (iliac and femoral). Moreover, experiments have been performed by the writer in which sounds were passed up the femoral artery into the aorta, whence pressures were obtained which were composed of the lateral pressure plus the velocity head. But even these aortic pressures are considerably less than

<sup>1</sup> Observations made by the writer with the Hürthle manometer show that in branches of the axillary there is a marked decrease in the systolic pressure, while the mean and diastolic pressures in these branches certainly do not differ much from the corresponding lateral pressures in the aorta. As quantitative values could not be given to these readings, they have been omitted from the table and charts.

those obtained in the iliac artery and *a fortiori* than those in the femoral artery.<sup>1</sup>

5. It is also noteworthy that the diastolic end pressure in the femoral is slightly, but distinctly and invariably, lower than that in the carotid artery, the relation of the femoral to the carotid diastolic pressure being as 95 to 103 (see section on the standard arteries, p. 250).

6. Erlanger has shown<sup>2</sup> that his sphygmomanometer records diastolic lateral pressures in the artery under examination, but systolic end pressures in the same artery. The conclusion<sup>3</sup> reached by Erlanger on purely *a priori* grounds, namely, that the pressures determined in the brachial artery by means of his instrument may be regarded as equivalent to the lateral pressures in the aorta at the origin of the subclavian, is almost certainly correct. His conclusion is entirely correct in the dog, and since the left subclavian system in the human being is essentially similar to that in the dog, there is no reason to doubt that Erlanger's instrument gives readings, when applied to the human brachial, which are equivalent to the lateral pressures in the aorta at the origin of the subclavian artery.

In the foregoing article it has been shown that the average mean carotid end pressure is greater than the corresponding average pressure in the femoral. In view of a statement recently published by Hürthle,<sup>4</sup> it seems desirable to add that in those experiments in which the mean end pressures of the carotid and the femoral were compared, the former had in every instance a slightly higher (1 to 4 mm.) value than the latter.

<sup>1</sup> It is an old observation made by HÜRTHLE with his elastic manometer, that the systolic end pressure in the femoral is greater than that in the carotid (*Archiv für die gesammte Physiologie*, 1890, xlvii, pp. 32-34). An explanation of the phenomenon has been offered (VON KRIES, *Studien zur Pulslehre*, Freiburg, 1892, pp. 67-68), but seems not to be entirely satisfactory. At present, however, no new explanation will be attempted.

<sup>2</sup> ERLANGER: A new instrument for determining the minimum and maximum blood pressures in man, Johns Hopkins Hospital reports, 1904, xii, p. 53.

<sup>3</sup> ERLANGER: *Loc. cit.*, p. 110, conclusion f.

<sup>4</sup> HÜRTHLE states (*Archiv für die gesammte Physiologie*, 1905, cx, p. 435) that in observations made upon fourteen animals (dogs, cats, and rabbits) the mean end pressure in the carotid was always slightly higher than that in the femoral. The experience of the present writer is, therefore, in complete accord with the results reported by HÜRTHLE.

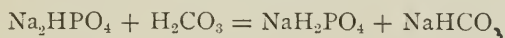
## EQUILIBRIUM IN SOLUTIONS OF PHOSPHATES.

By LAWRENCE J. HENDERSON.

[From the Laboratory of Biological Chemistry in the Harvard Medical School.]

TO phosphates wholly or chiefly the acidity of the urine has long been attributed; in blood, particularly in the red corpuscles, the presence of phosphates has been counted important in the transfer of carbonic acid, and though the functions of salts of phosphoric acid in living protoplasm, which is very rich in them, have not been made clear, no doubt exists that here they play an important rôle.<sup>1</sup> Accordingly, a careful analysis of the conditions of equilibrium in phosphate solutions is not without importance for biochemistry.

Before the introduction of the modern theory of solution and the concentration law, facts concerning equilibrium in solutions were necessarily stated otherwise than they are to-day, and conceptions regarding such systems were inevitably somewhat befogged. Nevertheless, the older literature contains not a little significant information regarding the nature of solutions of the salts of phosphoric acid, some of which is physiologically important. Berzelius himself indicated the nature of the reaction between di-sodium phosphate and carbonic acid, which we to-day write



and J. Setschenow<sup>2</sup> later showed that when a dilute solution of di-sodium phosphate, after being saturated with carbonic acid, is treated with barium chloride, no precipitate results. Here already the important fact that mono-sodium phosphate is an extraordinarily weak acid, in that it does not under certain circumstances decompose sodium bicarbonate, is clearly indicated.

<sup>1</sup> LOEW: Biologisches Centralblatt, 1891, xi, pp. 269-281.

<sup>2</sup> SETSCHENOW: Centralblatt für die medicinischen Wissenschaften, 1875, pp. 35-36.

By the observations of A. Joly<sup>1</sup> the understanding of mixed solutions of  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$  was materially advanced. He showed that, in the titration of phosphoric acid with sodium hydrate, helianthine and similar indicators serve to mark the formation of monosodium phosphate, and phenolphthalein the formation of di-sodium phosphate, whereas, as is well known, all these indicators mark almost precisely the same point in the titration of a strong acid with a strong base. Evidently, then, di-sodium phosphate is a very weak base.

In the year 1876 Maly proved<sup>2</sup> that when a neutral mixture of  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$  is subjected to dialysis the acid substance diffuses out more rapidly than the alkaline one, so that by repeated dialyses it is possible to separate the two substances quantitatively. Upon this fact he based the explanation of urinary acidity. Later<sup>3</sup> he carried out a more extended series of similar experiments on the diffusion of mixed salts, which again resulted in the separation of acid and alkaline substances. In these he placed mixtures of  $\text{NaCl} + \text{NaH}_2\text{PO}_4$ ,  $\text{CaCl}_2 + \text{NaH}_2\text{PO}_4$  and of other substances in the bottom of a cylinder with pure water above and awaited the result of diffusion. Maly's theoretical explanation of these phenomena, incorrect both through false reasoning and ignorance of the ionization hypothesis though it is, leads him to conclusions which are surprisingly near those which we hold to-day, and are accordingly of no little historical interest. Thus one reads: "I conceive that in blood serum free hydrochloric acid must be present quite as surely as free carbonic acid, fatty acid, lactic acid, uric acid, and acid monosodium phosphate."<sup>4</sup>

At the same time he insisted upon the importance of the reaction of carbonic acid with di-sodium phosphate in blood, maintaining that acid sodium phosphate must be present in blood notwithstanding the alkalinity, which was of course then accepted; he further indicated that, like carbonic acid, the other inorganic and organic acid products of metabolism must enter into reaction with di-sodium phosphate.

To Maly, then, the credit is due of having pointed out the importance to physiology of the more rapid diffusion of acid substances

<sup>1</sup> JOLY: Comptes Rendues, c, pp. 55-57.

<sup>2</sup> MALY: Berichte der deutschen chemischen Gesellschaft, 1876, ix, p. 164.

<sup>3</sup> MALY: Zeitschrift für physiologische Chemie, 1877, i, p. 174.

<sup>4</sup> "Ich denke mir im Blutserum ebenso freie Salzsäure, als darin freie Kohlen säure, Fettsäure, Milchsäure, Harnsäure und saures mononatrium phosphat vorhanden sein muss." *Loc. cit.* p. 187.



than of alkaline ones, and of insisting upon the theory that in blood plasma all the inorganic constituents are in equilibrium with one another.

In 1877 it was, of course, impossible for him correctly to analyze the factors which determine the nature of this equilibrium and the resulting concentration of hydrogen and hydroxyl ions.

With the introduction of physico-chemical habits of thought and physico-chemical methods into physiology, discussions of reaction and of equilibrium have been clarified, and as a result such discussions have multiplied.

Particularly extensive have been the investigations of the degree of acidity and alkalinity of animal fluids. As a result of the investigations of Höber,<sup>1</sup> Farkas,<sup>2</sup> P. Fränkel,<sup>3</sup> and Friedenthal,<sup>4</sup> we now know that blood, lymph, and protoplasm are almost precisely neutral; that is to say, they contain hydrogen and hydroxyl ions in almost equivalent concentrations. We know, too, that the actual acidity of urine is very slight, according to Höber's investigations<sup>5</sup> corresponding to only about  $0.5 \times 10^{-5} \overset{+}{\text{H}}$  ions per litre.

Friedenthal<sup>6</sup> has indicated the importance of the reaction between acids and sodium bicarbonate in blood as another regulatory mechanism more important in plasma than that between carbonic acid and sodium phosphate.

Not less important than the neutrality of plasma and protoplasm is their ability to take up considerable quantities of strong acid or strong alkali without materially departing from the neutral reaction. Thus Friedenthal found that in one case 70 times, in another 40 times, as much NaOH had to be added to blood serum as to water to get a standard red coloration with phenolphthalein, and to get a standard coloration with methyl orange once 327 times and once 387 times as much HCl.<sup>7</sup> Friedenthal explains the resistance to alkali by the ability of proteids to bind alkali, the resistance to acid by its reaction with salts composed of strong bases and weak acids.

<sup>1</sup> HÖBER: *Archiv für die gesammte Physiologie*, 1900, lxxxii, p. 522; 1903, xcix, p. 572.

<sup>2</sup> FARKAS: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 551.

<sup>3</sup> FRÄNKEL: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 601.

<sup>4</sup> FRIEDENTHAL: *Zeitschrift für allgemeine Physiologie*, 1902, i, p. 56; 1904, iv, p. 44.

<sup>5</sup> HÖBER: *HOFMEISTER'S Beiträge*, 1903, iii, p. 525.

<sup>6</sup> FRIEDENTHAL: *Loc. cit.*

<sup>7</sup> *Verhandlungsberichte der berliner physiologischen Gesellschaft*, 1903, p. 550.

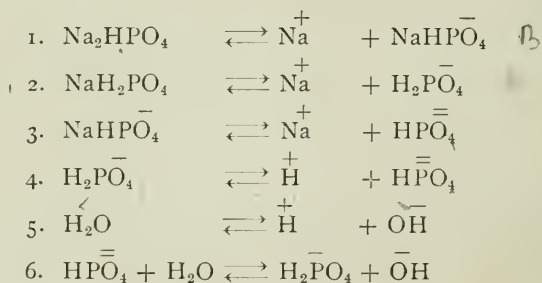


The time-honored idea that urine contains a mixture of mono- and di-hydrogen phosphates has recently been assailed by Folin<sup>1</sup> and by Dreser,<sup>2</sup> who maintain that only acid phosphates are there present.

Folin<sup>3</sup> bases his conclusions upon the fact that normal urine of acid reaction yields no precipitate of phosphates on the addition of calcium chloride.

Dreser<sup>4</sup> shows conclusively that the amount of titratable acidity of urine is far too much to be accounted for on the assumption that it is due exclusively to phosphoric acid in the salt  $\text{NaH}_2\text{PO}_4$ . On the basis of experiments with the extraction of anisic and salicylic acids from phosphate solutions and from urine with ether, he concludes that no di-sodium phosphate can be present in urine, but that a certain amount of free phosphoric acid, in addition to acid sodium phosphate exists there.

**Ionization in phosphate solutions.**—Theoretically the reactions of ionization and hydrolysis which occur in solutions of mixed mono- and di-sodium phosphates may be stated as follows:



Evidently the acidity or alkalinity of such a solution depends upon a number of factors; as the relative amounts of base and acid in the solution, which determine the relative amounts of mono- and di-sodium phosphates there present, the strength of the acid  $\text{NaH}_2\text{PO}_4$ , which determines the extent of the reactions (4) and (6), and the temperature, which in a rather high degree influences the ionization of water (5), as well as the other reactions of ionization, and so in a considerable degree affects the amount of hydrolysis.<sup>5</sup>

<sup>1</sup> FOLIN: This journal, 1905, xiii, p. 66.

<sup>2</sup> DRESER: HOFMEISTER'S Beiträge, 1905, vi, p. 178.

<sup>3</sup> FOLIN: *Loc. cit.* pp. 102-105.

<sup>4</sup> DRESER: *Loc. cit.*

<sup>5</sup> In case one has to deal with a weak base, as ammonium, the equilibrium is further complicated by the hydrolysis of the base.

The manner in which the acid mono-sodium phosphate and the alkaline di-sodium phosphate neutralize each other is somewhat peculiar, and in this, as in other similar cases, the mechanism is very important for the understanding of equilibrium in animal fluids. Hydrogen ions in excess of the quantity present in pure water can occur in such a solution only in accordance with the reaction (4). If they are present in such a solution, on the addition of di-sodium phosphate to it there is immediately formed an excess of the ion  $\text{HPO}_4^{=}$  according to the reactions (1) and (3) thereby immediately causing a decrease of the hydrogen ions in accordance with the concentration law by pushing back the reaction (4). On the other hand, if hydroxyl ions are present in such a solution as a result of hydrolysis, reaction (6), the addition of mono-sodium phosphate, by yielding the ion  $\text{H}_2\text{PO}_4^-$ , reaction (2), must, according to the concentration law, push back reaction (6), thus diminishing the concentration of the hydroxyl ions.

It is known that in a pure solution of the very weak acid  $\text{NaH}_2\text{PO}_4$  there are present very few  $\text{H}^+$  and  $\text{HPO}_4^{=}$  ions; accordingly the addition of very little di-sodium phosphate which yields much larger quantities of  $\text{HPO}_4^{=}$  ions, must very greatly decrease the hydrogen ionization of the solution. Similarly a pure solution of di-sodium phosphate contains few  $\text{OH}^-$  ions and a corresponding number of  $\text{H}_2\text{PO}_4^-$  ions, so that the addition of a small quantity of mono-sodium phosphate, which yields a great quantity of  $\text{H}_2\text{PO}_4^-$  ions, must greatly decrease the  $\text{OH}^-$  ionization of the solution.

Accordingly, we should expect most solutions of mixed  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$  to be nearly neutral, and this is, indeed, the case, as will be later explained.

Finally, inasmuch as hydrolysis proceeds, in the case of the phosphates, by the union of  $\text{H}^+$  ions with  $\text{HPO}_4^{=}$  ions, it is to be expected that with increasing temperature, and consequent increase in the active mass of  $\text{H}^+$  ions, hydrolysis should increase, in accordance with the concentration law; that is the more probable because the ionization of salts often decreases as the temperature rises.

In this connection it is not out of place to point out that the

difference between hydrolysis and ionization at room temperature and at body temperature is a matter that has not been adequately discussed by physiologists. It is not impossible, in view of the extreme sensitiveness of colloids to change in ionic concentration and to minute quantities of ions, that the increased ionization of water at  $38^\circ$  is one of the factors which makes that temperature favorable for physiological processes.

**Influence of temperature on equilibrium.**—The following experiments were carried out to determine the influence of temperature upon equilibrium in solutions of the phosphates and in certain animal fluids.

Equivalent solutions, approximately  $\frac{1}{10}$ , of  $\text{Na}_2\text{HPO}_4$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{NH}_4\text{OH}$ ,  $\text{NaOH}$  and  $\text{H}_2\text{SO}_4$  were prepared. With these standard solutions a series of titrations were made to determine at what points in the systems  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$ ,  $(\text{NH}_4)_2\text{HPO}_4 + \text{NH}_4\text{H}_2\text{PO}_4$ , urine +  $\text{NaOH}$ , urine +  $\text{H}_2\text{SO}_4$  at different temperatures the faintest possible coloration with phenolphthalein and methyl orange is to be observed. This method gives results which in any series of experiments are comparable among themselves, but depends for absolute values upon the concentration of the indicators and the degree of coloration chosen as a standard.

In the tabulated results the temperature is given in the first column, and beside it the quantity in c.c. of reagents which had to be added

PHENOLPHTHALEIN.								
Temp.	$\text{Na}_2\text{HPO}_4$ .	$\text{H}_3\text{PO}_4$ .	Temp.	$\text{Na}_2\text{HPO}_4$ .	$\text{H}_3\text{PO}_4$ .	Temp.	$\text{Na}_2\text{HPO}_4$ .	$\text{H}_3\text{PO}_4$ .
	c.c.	c.c.		c.c.	c.c.		c.c.	c.c.
$6^\circ$	50	0.9	$23^\circ$	50	1.4	$24^\circ$	50	1.5
$28^\circ$	50	1.6	$37^\circ$	50	2.0	$40^\circ$	50	2.4
$43^\circ$	50	2.1	$52^\circ$	50	3.3	$56^\circ$	50	3.4
$52^\circ$	50	2.5	$64^\circ$	50	4.2	$63^\circ$	50	4.2
$63^\circ$	50	3.2	$75^\circ$	50	5.1	$74^\circ$	50	4.6
$74^\circ$	50	4.2	$85^\circ$	50	6.2	$81^\circ$	50	5.4
$86^\circ$	50	5.3	$100^\circ$	50	7.4	$86^\circ$	50	5.9
$100^\circ$	50	6.7				$90^\circ$	50	6.3
						$100^\circ$	50	7.1

METHYL ORANGE.								
Temp.	Na <sub>2</sub> HPO <sub>4</sub> .	H <sub>3</sub> PO <sub>4</sub> .	Temp.	Na <sub>2</sub> HPO <sub>4</sub> .	H <sub>3</sub> PO <sub>4</sub> .			
24°	c.c. 50	c.c. 43.5	27°	c.c. 50	c.c. 43.5			
45°	50	45.5	41°	50	44.8			
57°	50	47.4	55°	50	47.1			
77°	50	54.2	62°	50	50.0			
100°	50	64.8.	77°	50	54.7			
27°	74	64.8	85°	50	58.1			
			100°	50	64.6			
METHYL ORANGE.								
Temp.	H <sub>3</sub> PO <sub>4</sub> .	NH <sub>4</sub> OH.	Temp.	H <sub>3</sub> PO <sub>4</sub> .	NH <sub>4</sub> OH.			
98°	c.c. 58.1	c.c. 25.0	88°	c.c. 56.9	c.c. 25.0			
78°	58.1	26.6	70°	56.9	27.0			
59°	58.1	27.8	40°	56.9	28.3			
42°	58.1	28.6	15°	56.9	28.9			
19°	58.1	29.2						
PHENOLPHTHALEIN.								
Temp.	H <sub>3</sub> PO <sub>4</sub> .	NH <sub>4</sub> OH *	Temp.	H <sub>3</sub> PO <sub>4</sub> .	NH <sub>4</sub> OH *	Temp.	H <sub>3</sub> PO <sub>4</sub> .	NH <sub>4</sub> OH *
98°	c.c. 25.0	c.c. 33	95°	c.c. 25.0	c.c. 34	86°	c.c. 25.0	c.c. 34
75°	28.5	33	70°	29.6	34	65°	29.7	34
60°	31.0	33	50°	32.0	34	53°	31.3	34
43°	32.7	33	36°	33.3	34	43°	32.5	34
14°	34.5	34	8°	34.5	34	29°	34.2	34
						17°	35.0	34
* Corrected for evaporation of ammonia.								

PHENOLPHTHALEIN.						
25 c.c. urine diluted with 50 c.c. water.						
Temp.	NaOH.	H <sub>2</sub> SO <sub>4</sub> .	Temp.	NaOH.	Temp.	NaOH.
6.0°	c.c. 12.1	c.c. 0.0	11°	c.c. 6.9	10°	c.c. 14.0
19.5°	13.1	0.0	27°	7.6	24°	14.9
33.0°	14.1	0.0	43°	8.8	37°	16.1
48.0°	15.5	0.0	62°	10.4	62°	18.7
61.0°	17.0	0.0	82°	12.6	78°	20.6
73.0°	18.4	0.0	96°	14.1	93°	22.6
85.0°	20.0	0.0				
98.0°	21.8	0.0				
12.0°	21.8	7.3				

METHYL ORANGE.					
25 c.c. urine diluted with 50 c.c. water.					
Temp.	H <sub>2</sub> SO <sub>4</sub> .	Temp.	H <sub>2</sub> SO <sub>4</sub> .	Temp.	H <sub>2</sub> SO <sub>4</sub> .
9°	c.c. 8.8	7°	c.c. 18.6	7°	c.c. 15.0
26°	10.0	29°	22.9	26°	16.7
46°	11.8	44°	25.6	45°	18.9
65°	13.6	59°	28.2	68°	21.6
79°	15.4	78°	31.7	86°	25.0
92°	17.9	94°	35.4		

to produce at that temperature the standard coloration with phenolphthalein or methyl orange as the case might be.

The following curves represent in the case of the salt solutions the ratios between the two constituents, base and acid, which at different temperatures are characterized by giving the faintest visible coloration with phenolphthalein or methyl orange, as the case may be; in the case of urine the titrable acidity (expressed in cubic centimetres  $\frac{N}{10}$  NaOH) or alkalinity (expressed in cubic centimetres  $\frac{N}{10}$  H<sub>2</sub>SO<sub>4</sub>) at different temperatures, in two characteristic experiments.

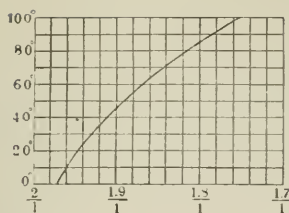


FIGURE 1. —  $\text{Na}_2\text{HPO}_4$ . Phenolphthalein. Abscissæ =  $\frac{\text{NaOH}}{\text{H}_3\text{PO}_4}$ .

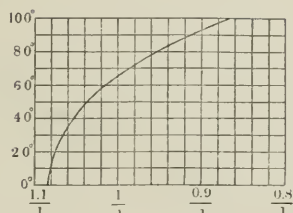


FIGURE 2. —  $\text{NaH}_2\text{PO}_4$ . Methyl orange. Abscissæ =  $\frac{\text{NaOH}}{\text{H}_3\text{PO}_4}$ .

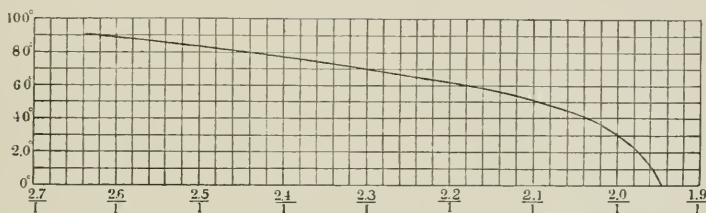


FIGURE 3. —  $(\text{NH}_4)_2\text{HPO}_4$ . Phenolphthalein. Abscissæ =  $\frac{\text{NH}_4\text{OH}}{\text{H}_3\text{PO}_4}$ .

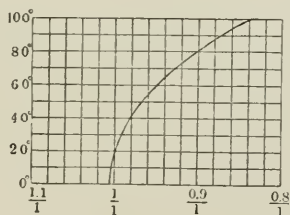


FIGURE 4. —  $\text{NH}_4\text{H}_2\text{PO}_4$ . Methyl orange. Abscissæ =  $\frac{\text{NH}_4\text{OH}}{\text{H}_3\text{PO}_4}$ .

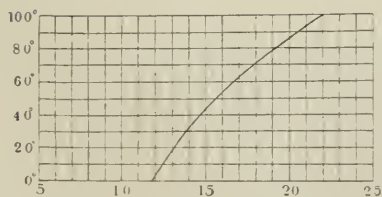


FIGURE 5. — Urine Phenolphthalein. Abscissæ are c.c.  $\frac{\%}{10}$  NaOH.

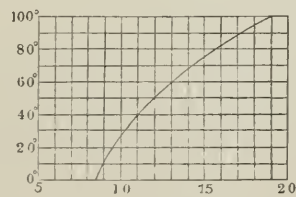


FIGURE 6. — Urine Methyl orange. Abscissæ are c.c.  $\frac{\%}{10}$   $\text{H}_2\text{SO}_4$ .



It appears from these experiments that temperature influences in a marked degree both the titratable acidity and alkalinity and the  $\text{H}^+$  and  $\text{OH}^-$  ionization of solutions of  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$ ,  $(\text{NH}_4)_2\text{HPO}_4 + \text{NH}_4\text{H}_2\text{PO}_4$  and urine, though in different ways. This influence must, according to our present theories, be dependent in high

PHENOLPHTHALEIN.									
25 c.c. urine + 16 gms. $\text{K}_2\text{C}_2\text{O}_4$ .									
Temp.	NaOH.	Temp.	NaOH.	Temp.	NaOH.	Temp.	NaOH.	Temp.	NaOH.
5°	c.c. 5.5	6°	c.c. 7.5	6°	c.c. 7.1	4°	c.c. 6.8	7°	c.c. 14.0
23°	6.6	25°	8.3	20°	7.8	20°	7.5	18°	15.1
		41°	8.9	32°	8.4	40°	8.3	34°	16.4
				45°	9.2	62°	9.3	55°	18.5
				75°	11.1	82°	10.4	70°	20.1
				100°	13.0	100°	12.1	98°	24.0

degree upon changing hydrolysis, which in turn is largely dependent upon changing ionization of water.

Accordingly, it seems just to draw the conclusion that hydrolysis is of primary importance in the equilibrium whereby the reaction of urine and of other body-fluids is established.<sup>1</sup>

In determining the titratable acidity of urine this effect of temperature is important, for unless the temperature is fixed errors of five per cent, and more, may readily be obtained. This is especially true when Folin's improved method is employed.

The determinations of urinary acidity were made precisely according to that method (see table above), except that the titration was first made at a low temperature and then at successively higher temperatures, as before.

In carrying out Folin's method the addition of potassium oxalate produces a fall in temperature of nearly ten degrees, so that results obtained in this way are always lower than would be the case if the titration occurred at room temperature.

<sup>1</sup> Blood serum acts like urine, though to a less marked degree. BAXTER has recently found that in titrating  $\text{Na}_2\text{CO}_3$  with  $\text{HCl}$ , using phenolphthalein as an indicator, *considerably* more acid must be added at a lower temperature than at a higher one. (Verbal communication.)

The following curve indicates the average influence of temperature upon urinary acidity determined by Folin's method as I have found it. I have indicated by two cross marks the acidity at room temperature (20°) and at the temperature which results in the mixture from the addition of potassium oxalate (12°); the difference is about five per cent; it may well be smaller in more acid urine.

Not without interest is the fact that urine and blood, like the ammonium phosphates and unlike the sodium phosphates, become on heating more acid to phenolphthalein and more alkaline to methyl orange. This is doubtless due to the presence of weak bases as well as weak acids in the solution, and weighs against the conclusion of Friedenthal.<sup>1</sup>

**Hydrogen ionization.** — Far more important than the influence of temperature upon equilibrium in

solutions of the phosphates is the actual  $\overset{+}{H}$  and  $\overset{-}{OH}$  ionization in such solutions, for as we now know protoplasm and body fluids are almost precisely neutral. Regarding this ionization data exist. It has been directly determined by Friedenthal<sup>2</sup> and may be calculated from the investigations of Boettger.<sup>3</sup> The latter, studying the use of the electrometer as an indicator for titration, obtained results which upon calculation give the  $\overset{+}{H}$  ionization in mixed solutions of mono- and di-sodium phosphates ( $\frac{n}{2}$ ) as represented in the following table in the first column. The results of Friedenthal determined directly with the aid of the concentration cell and with the aid of indicators

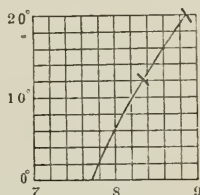


FIGURE 7. — Urine +  $K_2C_2O_4$ . Phenolphthalein. Abscissae are c.c.  $\frac{n}{10}$  NaOH.

$\frac{NaH_2PO_4}{Na_2HPO_4} =$	10 : 0	8 : 2	6 : 4	4 : 6	2 : 8	0 : 10
I.	$6.4 \times 10^{-5}$	$2.1 \times 10^{-6}$	$6.9 \times 10^{-7}$	$3.7 \times 10^{-7}$	$1.3 \times 10^{-7}$	$1.0 \times 10^{-8}$
II.	$3.3 \times 10^{-5}$	$1.5 \times 10^{-6}$	$4.9 \times 10^{-7}$	$1.9 \times 10^{-7}$	$6.5 \times 10^{-8}$	$1.3 \times 10^{-9}$
III.	$5.0 \times 10^{-5}$	....	....	....	....	$1.0 \times 10^{-9}$

<sup>1</sup> FRIEDENTHAL: "Bei den Flüssigkeiten in den Organismen beruht das Zustandekommen der Neutralität . . . auf der Neutralisation von starkem Alkali durch einen Ueberschuss von schwacher Säure." Zeitschrift für allgemeine Physiologie, 1904, iv. p. 57.

<sup>2</sup> FRIEDENTHAL: *Loc. cit.*

<sup>3</sup> Zeitschrift für physikalische Chemie, 1897, xxiv, p. 253.

are recorded in the second and third columns. The latter used  $\frac{2}{10}$  solutions.

These results are in exceedingly good accord, considering the difference in concentration of the solutions and the difference in method. It seems, however, preferable to make use of the results of Friedenthal for a physiological discussion, for they were obtained directly and in solutions more like the physiological phosphate solutions in concentration, while in Boettger's method there are errors, of no importance for his conclusions, which affect the absolute values calculated from his data.

Evidently in solutions of mixed mono- and di-sodium phosphates the hydrogen and hydroxyl ionization is always very small, as was to have been expected according to the theoretical discussion. Thus the organism and all living cells possess, in the abundant quantities of phosphates which they hold in solution, an efficient mechanism to prevent the occurrence of considerable hydrogen or hydroxyl ionization, that is to say, of even slight acidity or alkalinity. For it is evident that to a mixed phosphate solution enough strong alkali or acid to produce either exclusively mono- or di-sodium phosphate may be added without causing more than faint acidity or alkalinity.

If, for instance, hydrochloric acid be added to a solution of sodium phosphate, the reaction  $\text{Na}_2\text{HPO}_4 + \text{HCl} = \text{NaCl} + \text{NaH}_2\text{PO}_4$  takes place, and so long as the amount of HCl added is less than enough to convert all the di-sodium phosphate into mono-sodium phosphate the hydrogen ionization will correspond closely to that of the resulting mixture of the two phosphates and free hydrochloric acid will be absent from the solution, save for an infinitesimal amount, according to the well-known law of the distribution between two acids of a base.

It is true that the acidity of mono-phosphates and the alkalinity of di-phosphates lies somewhat outside of the range which we find in the cell. Nevertheless, between these two points there is a wide range of variation in the ratio of base to acid where the hydrogen and hydroxyl ion concentrations correspond to those which have been found for blood serum and tissues, — values well established as regards the average, though as yet we do not know what variations may normally occur in them.

For convenience, I give here a curve representing hydrogen ionization in  $\frac{2}{10}$  solutions of  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$ , according to Friedenthal, and I have there marked off the extreme range of hydrogen ionization which has been accurately observed in serum.

The existence of such a mechanism as this has long been argued with respect to the condition of carbonic acid in blood, according to the reaction first discussed by Berzelius.

However, with the aid of the dissociation hypothesis it is for the first time possible to perceive how much more general such reactions of phosphates with bases and acids must be and how completely they protect the organism from acid or alkaline substances.

Through this adjustment of equilibrium acid or alkali is immediately neutralized; the organism may then proceed at its leisure to produce ammonia for the readjustment of equilibrium at its proper level, provided enough acid is present to render that process desirable.

It is, of course, not to phosphates alone that this function of neutralization is entrusted. The equilibrium of hydrogen and hydroxyl ionization, as has quite sufficiently been explained in recent years, is one in which all salts of a physiological solution as well as the proteids are concerned. Thus in circulating blood plasma mixtures of  $\text{NaHCO}_3 + \text{H}_2\text{CO}_3$ , as is known,<sup>1</sup> aid in the performance of this function, yielding on the addition of acid a salt and more carbonic acid; on the addition of alkali more acid carbonate. In all body-fluids there are present other salts of weak acids and weak bases, together with the free acids and bases in very small amounts, and these too must enter into the reaction. Accordingly it is a mistake to regard the equilibrium whereby neutrality is maintained in blood and protoplasm as one between strong bases and weak acids.

The peculiar importance of phosphates in this respect rests, then, not upon a unique property of their solutions, but upon the facts that in all living protoplasm they are the chief saline constituents and that they are capable of exceptionally great variation in the ratio of base to acid with exceptionally little resulting variation in hydrogen

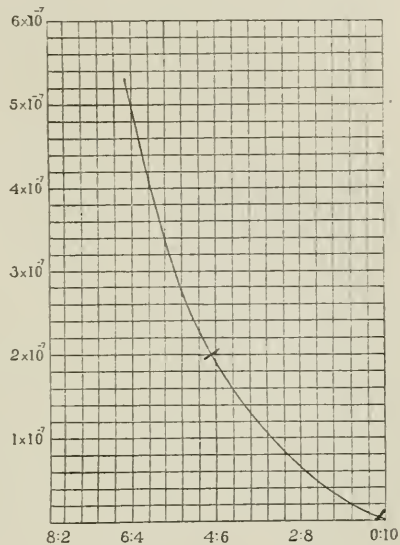
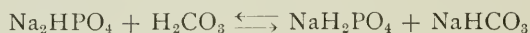


FIGURE 8.—Hydrogen ionization in sodium phosphate solutions. Abscissæ =  $\frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4}$

<sup>1</sup> FRIEDENTHAL: *Loc. cit.*

ionization, as above explained. How they share in this regulatory mechanism with the bicarbonates is clear from a consideration of the nature of the reaction



When, on the addition of acid to a solution of sodium phosphates and carbonates, the acidity of the phosphates has reached a certain point, the amount of bicarbonate present being probably little affected up to this point, the continued addition of acid must decompose the sodium bicarbonate. When the sodium bicarbonate is completely decomposed, the ratio between mono- and di-sodium phosphates, during the period of decomposition of sodium bicarbonate probably but slightly affected, again begins to change rapidly, and eventually the solution will contain only acid sodium phosphate and the sodium salt of the acid added.

Clearly one function of phosphates as a constituent of protoplasm is now established. In this respect Maly's diffusion experiments appear in a new light. By the processes of intracellular metabolism acids are produced in greater quantity than bases ( $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{CO}_3$ , etc.); thus, by the activity of the cell, acid potassium phosphate is being constantly increased at the expense of di-potassium phosphate. The acid potassium phosphate, diffusing more rapidly than di-potassium phosphate, reaches the cell wall more rapidly than that substance, provided the cellular mechanism does not overcome the physical tendency, and thus acid may be conveniently removed from its point of origin.

**Equilibrium in urine.** — That the view of Folin and Dreser regarding phosphate equilibrium in urine is true seems to me improbable. The argument of Folin that urine contains no di-sodium phosphate because it gives no precipitate with calcium chloride is fallacious in that a mixture of  $\text{NaH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$  which contains enough of the former substance gives no precipitate on the addition of calcium chloride, while the drawing of conclusions regarding quantitative relationships, as Dreser has done, from experiments with a pure salt solution on the one hand and a complicated mixture like urine on the other is not without danger. The determinations of hydrogen ionization in urine and its behavior toward indicators both support the view that in urine there exists a mixture of mono- and di-hydrogen phosphates of sodium, ammonium, and other bases. Thus Höber has found the hydrogen ionization of normal urine to be less than



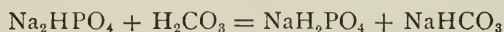
$1 \times 10^{-5}$  in all cases, while that of acid sodium phosphate is more than  $3 \times 10^{-5}$  according to all determinations, and urine gives no coloration with methyl orange, but acid sodium phosphate gives a distinct acid reaction with that indicator, a reaction not diminished by the addition of a neutral sodium salt, as sodium chloride, or of urea; the latter fact seems to me very difficult to explain otherwise than by the presence of "neutral" phosphates in urine.

#### SUMMARY.

The titratable acidity of urine varies materially with the temperature, partly because of the variation in the ionization of water with the temperature. This variation should be regarded in practical work, for it may produce errors of more than five per cent.

The hydrogen ionization of blood serum corresponds to that of solutions of mixture of  $\text{Na}_2\text{HPO}_4 + \text{NaH}_2\text{PO}_4$  in which the ratio of the two constituents varies between 6:4 and 1:0 approximately. Variation within this range being presumably harmless, protoplasm possesses in the phosphates, aided by other substances in less degree, a mechanism whereby great quantities of acid or alkali may be immediately neutralized and the hydrogen ionization preserved within normal limits.

The reaction



is a balanced one, and even in solutions less acid than mono-sodium phosphate sodium bi-carbonate cannot exist.

Both the determinations of hydrogen ionization in urine and in phosphate solutions, and their behavior to methyl orange indicate that urine contains a mixture of mono- and di-hydrogen phosphates.



## A PRELIMINARY STUDY OF THE CHEMISTRY OF NERVE TISSUE DEGENERATION.

BY WALDEMAR KOCH AND WILLIAM H. GOODSON.<sup>1</sup>

[From the Laboratory of, *Physiological Chemistry of the University of Missouri, Columbia, Mo.*]

AMONG previous investigations into the chemical composition of normal and degenerated nerve tissues may be mentioned those of Gutnikov,<sup>2</sup> Noll,<sup>3</sup> Mott and Barratt,<sup>4</sup> Barratt,<sup>5</sup> and Mott and Halliburton.<sup>6</sup> None of these investigators attempted the separation of the several chemical individuals of the nervous system, but determined the amount of water and usually also the total phosphorus. The various points of interest in the above publications will be mentioned in the body of the paper as they happen to bear upon some point of this investigation.

The methods of collecting material and of chemical analysis were essentially those devised by one of us.<sup>7</sup> More recent publications suggest the following changes:

**Determination of lecithans.** — In place of the rather tedious method previously devised, the more elegant one based on the separation as lead salts, first suggested by Thudichum<sup>8</sup> and improved by H. S. Woods,<sup>9</sup> was used.

<sup>1</sup> Research scholar of the Rockefeller Institute for Medical Research.

<sup>2</sup> GUTNIKOV: *Zeitschrift für Psychiatrie*, 1897, liii, p. 270.

<sup>3</sup> NOLL: *Zeitschrift für physiologische Chemie*, 1899, xxvii, p. 370.

<sup>4</sup> MOTT and BARRATT: *Proceedings of the Physiological Society*, London, 1899, xxiv, p. 111; *Archives of neurology*, 1899, i, p. 350.

<sup>5</sup> BARRATT W.: *Archives of neurology*, 1899, i, p. 207.

<sup>6</sup> MOTT and HALLIBURTON: *Philosophical transactions of the Royal Society*, 1901, cxciv, B, p. 437.

<sup>7</sup> KOCH: *This journal*, 1904, xi, p. 303.

<sup>8</sup> THUDICHUM: *Die chemische Konstitution des Gehirns des Menschen und der Tiere*, 1901, p. 282.

<sup>9</sup> KOCH and WOODS: *Journal of biological chemistry*, 1905, i, p. 203.

As in the case of nerve tissue a sulphur compound which contains phosphorus follows into the lecithin and kephalin portions, it is necessary to make both sulphur and phosphorus determinations. For this purpose fusion with sodium carbonate and potassium nitrate (7 : 1) or sodium peroxide over an alcohol lamp is made use of. This fused mass, after dissolving, is acidified with nitric acid, and barium nitrate solution (1 per cent), added for the sulphur estimation. By this procedure no further difficulty is experienced in determining phosphorus by the molybdate method. One molecule of phosphorus for every two molecules of sulphur must then be subtracted from the phosphorus result, and lecithin and kephalin calculated respectively from the remainder as directed in the publication of the method above referred to.

**Determination of cerebrins.** — The recent work of Thierfelder<sup>1</sup> confirming the older results of Thudichum has given us a formula with which, as a basis, can be calculated theoretically the amount of cerebrin corresponding to the amount of reducing sugar found. Unfortunately Thierfelder does not give the factor by which he has calculated the amount of galactose from his titration, so that exact comparison with the table given in the paper by one of us above referred to is not possible. A calculation with the factor 0.4535 for CuO gives 19.5 per cent galactose as compared with Thierfelder's 21.8 per cent. In spite of this discrepancy it is probable, however, that the lower result corresponds more closely with the quantity usually split off under the ordinary conditions given in the directions. The figures given in the table of the first paper were therefore used in the calculations of this paper.

It may also be well at this point to again call attention to the importance of adding a large excess of Fehling's solution to reduce the sugar. If at any time the solution acquires a greenish tint, not enough has been added. Sometimes white flakes appear in the solution mixed with the brick red precipitate; these indicate that solution B is too old and must be renewed. In no case is it safe to make quantitative estimations with solutions made up ready for use in which such substances as glycerine or mannite are used, as they always give a reduction on their own account.

**Determination of cholesterin.** — Buenz<sup>2</sup> in Thierfelder's laboratory has recently determined that cholesterin does not exist in the ner-

<sup>1</sup> THIERFELDER: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 366.

<sup>2</sup> BUENZ: *Zeitschrift für physiologische Chemie*, 1905, xlvi, p. 47.

vous system in the form of esters, but in the free state, and it should be reported as such.

**Determination of sulphur compound.**—More recent investigation<sup>1</sup> suggests that the sulphur compound exists in two forms,—combined with the lipoids so as not to be removed by dilute acids, and free in the watery extract. Whether the two are identical has not yet been determined. The compound so far analyzed is the one soluble in water and not combined with the lipoids (lecithin, kephalin, cerebrin). For the present, therefore, it is necessary to determine both, and to calculate the combined one on the basis of the composition determined for the soluble one, namely, sulphur 4 per cent and phosphorus 2 per cent. It is necessary in determining the extractive sulphur, which is represented mainly by the uncombined compound, to use a fusion mixture, as ignition without the presence of an excess of alkali leads to loss of sulphur. The inorganic sulphates must also be determined and corrected for.

The tissues analyzed in the course of this investigation included three normal brains, three brains from cases of general paralysis of the insane, and the spinal cords of two dogs,—one normal and the other degenerated. The results of the analyses of the normal tissues will be first discussed.

Following is the record of the normal brains:

**Case 1 (VI).**—London. F. 03. Age 28. Autopsy twenty-eight hours after death. Weight of brain 1150 gms. No wasting, slightly œdematous (also observed on microscopic examination kindly made by Dr. G. Watson).

*Cause of Death.*—Shock from operation for intestinal obstruction.  
*Mental State.*—Normal.

**Case 2<sup>2</sup> (VIII).**—Columbia. M. 04. (Negro.) Age 20. Autopsy twenty-four hours after death. Weight of brain (not taken) about 1400 gms. No wasting, no excess of fluid.

*Cause of Death.*—Infection of *Bacillus capsulatus ærogenes*, introduced by gunshot wound. *Mental State.*—Normal.

**Case 3<sup>2</sup> (IX).**—Columbia. M. 04. Age 35. Autopsy three hours after death. Weight of brain 1425 gms. No wasting. No excess of fluid under membranes.

*Cause of Death.*—Not known. Death came very suddenly in hospital. *Mental State.*—Normal. Carpenter by profession.

<sup>1</sup> KOCH: Science, 1905, xxi, p. 884.

<sup>2</sup> These two cases were secured through the kindness of Prof. W. McNAB MILLER of the University of Missouri.

The analytical figures of the above cases show great variations which will be given in detail at the end. The average results, calculated in per cent of total solids, are here given for the various parts of the nervous system analyzed.

TABLE I.

	Prefrontal area.	Motor area.	Corpus callosum.	Sciatic nerve.
Total solids . . . . .	17.5	18.4	30.0	35.8
Simple proteids . . . . .	25.8	26.6	16.7	12.6
Nucleo-proteids . . . . .	23.8	21.0	11.4	34.9
Lecithins . . . . .	14.8	12.4	14.5	7.1
Kephalins . . . . .	8.9	11.0	7.6	7.8
Cerebrins . . . . .	7.1	8.6	17.7	7.2
Sulphur compound (combined)	5.9	5.9	7.3	10.0
Extractives . . . . .	11.0	10.6	5.8	5.0
Inorganic salts . . . . .	7.0	6.0	2.7	3.6
Number of analyses making up the average are, in case of prefrontal, 5; motor, 5; corpus callosum, 4; sciatic nerve, 1.				

The following conclusions may be drawn from the results in Table I:

1. The gray matter of the prefrontal and motor area differs very little in composition. The differences are within the limits of error.
2. The corpus callosum, representing a mass of medullated nerve fibres of the central nervous system, differs markedly from the gray matter. The amount of proteids is less and the amount of cerebrin greater. The lecithin and kephalin are very nearly the same as for the gray matter, but relatively to the proteids they are increased, although not so much as the cerebrins, which according to Noll are found only in medullated fibres. The relation of lecithins and kephalins to one another shows no change that cannot be accounted for by uncertainties in the method, which, at the time some of these analyses were made, had not been so well worked out. The extractives and inorganic salts are markedly less than in the gray matter when calculated in per cent of total solids, but not with calculations made for moist tissues.

3. Comparison of the corpus callosum and sciatic nerve reveals the fact that the two differ markedly in composition. The larger amount of the proteids may be accounted for by the greater amount of connective tissue in the sciatic nerve, a conclusion that is confirmed by the higher result for the nucleo-proteid and the lower result for water.

The result for the nucleo-proteid is almost sure to be too high, as the phosphorus content of the nucleo-proteid isolated from the central nervous system was used as a basis for calculation, which nucleo-proteid has an unusually low per cent of phosphorus. The amounts of lecithin, kephalin, and cerebrin are not actually so small as they appear to be, as the per cent is lowered by including among the total solids in the calculation the large amount of connective tissue proteids. Connective tissue contains extremely small amounts of lecithin and kephalin. It is interesting to note, however, that relative to the lecithin and kephalin the amount of cerebrin is decreased, so that the medullated fibres of the central nervous system would appear to contain proportionately more of this substance than peripheral medullated fibres. The inorganic salts and extractives are very nearly the same.

4. It will be noticed that the amount of the sulphur compound found combined with the lipoids is greater in the corpus callosum than in the gray matter, and even greater in the sciatic nerve than in the corpus callosum. The significance of this may be revealed when we know more about this interesting compound. The soluble sulphur compound is here included among the extractives. No conclusions can be drawn here with regard to it, as it had not been fully investigated when these analyses were made.

For the pathological material cases of general paralysis of the insane were chosen, as these cases show, both macroscopically and microscopically, the greatest amount of change, and should give the greatest variation from the normal, if such variations can be demonstrated by chemical methods at all.

The cases, collected at the Pathological Laboratory of the London County Asylums, Claybury, through the kindness of Dr. F. W. Mott, are as follows :

**Case 4 (II).** — Claybury. 24. M. 032. Age 27. Autopsy twenty-four hours after death. Weight of brain 1215 gms. Some general wasting. Slight excess of fluid.



*Cause of Death.* — Heart failure and hypostatic congestion of lungs.  
*Mental State.* — Early general paralysis.

**Case 5 (IV).** — Claybury. 38. M. 032. Age 36. Autopsy four hours after death. Weight of brain 1315 gms. Very marked wasting. Considerable excess of fluid.

*Cause of Death.* — Broncho-pneumonia. *Mental State.* — General paralysis with considerable dementia.

**Case 6 (VII).** — Claybury. 42. M. 032. Age 33. Autopsy thirteen hours after death. Weight of brain 1180 gms. Marked general wasting. Large excess of fluid.

*Cause of Death.* — Lobar pneumonia. *Mental State.* — General paralysis with much dementia.

In the following table the average results of these three cases are compared with the average results of the three normal cases. This method of comparing averages of several cases, even when as typical and hence comparable as the above, is not free from objection. A consideration of the table at the end, however, indicates that a comparison of the maximum and minimum results leads to the same conclusions as the averages. Whenever the attempt is made to separate the gray from the white matter, the variations in the analytical results are liable to be considerable, as Gutnikov's figures also indicate. The results of an experimental degeneration are also given for comparison (see Table II.).

The following conclusions may be drawn from Table II.

1. The degenerated nerve tissues contain less solids than the normal, due to the fact that, as the cortex wastes away, cerebro-spinal fluid partially takes its place and renders the tissues more watery. Barratt mentions the same observation in his paper. A certain amount of œdema may also play a part in rendering the tissues more watery.

2. The nucleo-proteids are increased, due mainly to the presence of large numbers of leucocytes, proliferating blood vessel elements, and neuroglia cells.

3. The average and the maximum and minimum results indicate little or no change in lecithins, kephalins, and sulphur compound (combined), in the prefrontal as well as the motor areas, which cannot be accounted for by variations in the material. The increased amount of cerebrin in the motor area (degenerated) indicates that these samples contained a larger admixture of white matter.

4. The experimental degeneration produced by cutting the cord of a dog and allowing it to degenerate for nineteen days gives results



on chemical analysis which resemble the degeneration of general paralysis. There is a similar increase in the amount of water and nucleo-proteid, and comparatively little change as regards the relative amounts of the other constituents.

5. Barratt's interesting observation that the percentage of total phosphorus in normal and degenerated brains is the same, is here

TABLE II.

	Prefrontal area.		Motor area.		Spinal cord of dog.	
	Normal.	Degenerated.	Normal.	Degenerated.	Normal.	Degenerated.
Total solids . . . . .	17.5	15.2	18.4	17.4	31.5	28.4
Simple proteid . . . . .	25.8	19.1	26.6	12.1	18.1	14.9
Nucleo-proteid . . . . .	23.8	37.7	21.0	35.3	6.7	12.8
Lecithins . . . . .	14.8	12.3	12.4	15.0	} 26.4	26.3
Kephalins . . . . .	8.9	8.5	11.0	15.0		
Cerebrins . . . . .	7.1	7.7	8.6	12.0	15.9	15.1
Sulphur compound (combined)	5.9	5.6	5.9	6.0	5.3	6.4
Extractives . . . . .	11.0	10.8	10.6	10.0	3.7	3.4
Inorganic salts . . . . .	7.0	6.6	6.0	6.0	2.2	2.0

only partially confirmed. The relative amounts of the alcohol, ether, soluble phosphorus compounds, the lecithins and kephalins are indeed practically unchanged, although their absolute amount is much reduced. The interesting increase in nuclein phosphorus entirely escaped his notice.

6. The absolute amounts of lecithins, kephalins, cerebrins, and sulphur compound must be very much reduced in general paralysis; their proportion relative to one another remains, however, practically unchanged.

7. In conclusion, it is interesting to note that the nervous system more than any other tissue, both in pathological and experimental degeneration, tends to keep its relative composition constant, which observation is in harmony with the results obtained in starvation.

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TABLE III.

	PREFRONTAL (HUMAN).						MOTOR (HUMAN).						CORD (DOG).	
	Normal.			Degenerated.			Normal.			Degenerated.			Normal.	Degen-erated.
	Max.	Min.	Av'ge.	Max.	Min.	Av'ge.	Max.	Min.	Av'ge.	Max.	Min.	Av'ge.		
Water . . . . .	83.0	82.2	82.5	85.3	84.3	84.8	82.3	80.2	81.6	84.5	80.6	82.6	68.5	71.6
Simple proteid . . . . .	5.8	2.8	4.5	5.7	0.3	2.9	7.2	3.8	4.9	3.7	0.8	2.1	5.7	4.2
Nucleo-proteid . . . . .	5.5	3.1	4.0	8.4	3.0	5.7	5.4	1.8 <sup>+</sup>	3.9	7.4	4.8	6.1	2.1	3.7
Lecithins . . . . .	3.2	2.1	2.6	3.1	1.3	1.9	3.0	1.8	2.3	3.3	1.6	2.6	} 8.3	7.5
Kephalins . . . . .	2.3	1.1	1.6	2.2	0.9	1.3	2.7	1.5	2.0	3.0	1.9	2.6		
Cerebrins . . . . .	1.8	0.7	1.3	1.4	1.0	1.2	2.0	1.3	1.6	2.4	1.6	2.1	4.3	4.3
Sulphur compound (combined)	1.6 <sup>+</sup>	0.6	1.0	1.1	0.7	0.9	1.3	1.0	1.1	1.5 <sup>+</sup>	0.7	1.1	1.7	1.8
Extractives . . . . .	2.2	1.7	1.9	2.0	1.1	1.7	2.7	1.3	2.0	..	..	1.7	1.2	1.0
Inorganic salts . . . . .	1.3	1.0	1.2	1.2	0.8	1.0	1.2	0.9	1.1	..	..	1.0	0.7	0.6

+ Analyses marked with a cross are probably incorrect, but no definite cause could be found for excluding them.

# VAGUS INHIBITION OF THE HEART IN ITS RELATION TO THE INORGANIC SALTS OF THE BLOOD.

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IT is well known that an increase in the percentage of the neutral potassium salts, especially the potassium chloride, present in the blood or other circulating medium causes the heart to beat at a slower rate and eventually to come to rest in a condition of diastole, with loss of tone, just as in the case of vagus inhibition.<sup>1</sup> It is known also that this condition of potassium arrest is not the result of an immediately toxic action of the potassium salts on the tissues of the heart, since two observers, at least, have shown that simple removal of the excess of potassium salts, by diffusion for example, is followed by a recovery of the power of the heart to develop its normal rhythmic contractions.<sup>2</sup> The resemblances between the conditions of potassium inhibition and vagus inhibition have been noted by several observers,<sup>3</sup> and have led some to suggest that the two phenomena are essentially identical.

On the other hand it has been shown by a number of observers, in experiments made chiefly on the hearts of cold-blooded animals, that an increase in the calcium salts in the circulating liquid leads to an augmented tonicity, an increased force and duration of the systolic contraction, and at times to an accelerated rate of beat.<sup>4</sup> In

<sup>1</sup> For literature see BRAUN: *Archiv für die gesammte Physiologie*, 1904, ciii, p. 476. and HALD: *Archiv für experimentelle Pathologie und Pharmakologie*, 1905, liii, p. 227.

<sup>2</sup> MARTIN: *American journal of physiology*, 1904, xi, p. 370; BRAUN: *Loc. cit.*

<sup>3</sup> BOTTAZZI: *Archives de physiologie*, 1896, p. 882; HOWELL: *This journal*, 1901, vi, p. 204; MARTIN: *Loc. cit.*

<sup>4</sup> RINGER: *Journal of physiology*, iv, p. 29; v, 247; 1895, xviii, p. 425, etc.; HOWELL: *Loc. cit.*; LANGENDORFF and HUECK: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 473.

view of these facts the author was led to suggest some years ago that the inhibitory influence of the vagus and the augmenting influence of the sympathetic upon the heart may be exerted through some intermediate effect upon the potassium and calcium compounds in the heart tissue. With this general idea in mind an attempt has been made to determine experimentally to what extent the inhibitory influence of the vagus on the heart is affected by variations in the potassium and calcium contents of the circulating medium. The results of these experiments are reported in this paper.

The experiments were made upon terrapins and frogs. The heart was isolated, although left *in situ*. It was kept beating by means of a standard Ringer's solution which was supplied at constant pressure from a reservoir. The inflow cannula was inserted into one of the large veins opening into the heart, the other veins being ligated,<sup>1</sup> while the outflow from the ventricle was received through a cannula which was inserted into one of the aortæ and pushed through the valves so that its end lay freely in the ventricular cavity. In some cases the arterial cannula was omitted, the ventricle being allowed to empty itself through the stumps of the severed arteries. The contractions of the ventricle and the left auricle were recorded in most of the experiments, but in some cases registration was omitted in order to keep the heart free from the mechanical tension of the levers.

#### I. EFFECT OF VARYING THE AMOUNT OF POTASSIUM SALT IN THE CIRCULATING LIQUID.

The standard Ringer's solution used to irrigate the heart in this series of experiments had the following composition :

Sodium chloride	0.7	per cent.
Calcium chloride	0.023	" "
Potassium chloride	0.03	" "

The experiments were made upon terrapins. After the heart had been isolated and was beating regularly on the standard solution, it was submitted to the action of a series of liquids in which the percentage of potassium chloride varied from 0.01 per cent to 0.10 per cent, while the sodium and the calcium chloride were kept constant at

<sup>1</sup> Great care must be exercised in ligating the right superior cava in the terrapin, to avoid injury to the cardiac branches of the vagus. The ligatures must be laid as far as possible from the heart.

the percentage of the standard Ringer's mixture. The effect of these different circulating mixtures upon vagus inhibition was determined by ascertaining the least strength of stimulus capable of giving a perceptible effect upon auricle or ventricle in the direction of either a slowing of rate or a weakening of the beat. The stimulus used was a tetanizing induction current applied to the vagus nerve in the neck. The strength of the stimulus was expressed in terms of the graduation upon the coil (Gaiffe). In the terrapin the right vagus was usually more effective, in the frog, the left vagus. Such results as the following were obtained:

1. On standard Ringer's mixture (potassium chloride 0.03).
  - Minimal inhibition, auricle (force) . . . . . 150
  - "    "    ventricle (rate) . . . . . 200
  - On Ringer's mixture with potassium chloride 0.05-0.07.
  - Minimal inhibition, auricle (rate) . . . . . 100
  - "    "    ventricle " . . . . . 100-150
2. Standard mixture (KCl 0.03).
  - Minimal inhibition, auricle (rate) . . . . . 500-600
  - "    "    ventricle " . . . . . 500-600
  - Ringer's mixture with potassium chloride 0.05-0.07.
  - Minimal inhibition, auricle (rate) . . . . . 300
  - "    "    ventricle " . . . . . 300
3. Standard mixture (KCl 0.03).
  - Minimal inhibition, auricle (rate) . . . . . 200
  - "    "    "    (force) . . . . . 150
  - "    "    ventricle (rate) . . . . . 200
  - Ringer's mixture with potassium chloride 0.05.
  - Minimal inhibition, auricle (rate) . . . . . 150
  - "    "    ventricle " . . . . . 150
  - Ringer's mixture with potassium chloride 0.06.
  - Minimal inhibition, auricle (rate) . . . . . 100
  - "    "    ventricle " . . . . . 100
4. Standard mixture (KCl 0.03).
  - Minimal inhibition. The heart not affected at all by strengths  
     of current to . . . . . 600 or 700
  - Ringer's mixture with potassium chloride 0.07.
  - Minimal inhibition, auricle (rate) . . . . . 150
  - "    "    ventricle " . . . . . 150

*These and similar results show very clearly that an increase in the concentration of the potassium salts within certain limits increases the sensitiveness of the heart to vagus stimulation. This effect was*

observed most distinctly in those hearts which for some reason exhibited an initial insensitiveness to vagus stimulation while under irrigation with the standard Ringer's mixture. In a number of cases hearts were found which before the operation were easily inhibited by stimulation of the vagus, but after isolation and while under irrigation with the standard mixture were inhibited with difficulty, that is, even maximal stimulation of the vagus caused only a slight slowing of the beat, or a short pause followed by a slowed beat. In such cases a slight increase in the potassium percentage made the heart very responsive to the action of the vagus, relatively slight stimuli causing complete diastole in all chambers for long periods.

This favorable influence of the potassium salts has, however, a distinct limit. When the percentage of the potassium chloride is increased to a point, 0.10 per cent or higher, at which it affects distinctly not only the rate, but the force or sequence of the heart-beats, the action of the vagus instead of being favored is markedly depressed. This result is observed in the phase just preceding the appearance of complete potassium arrest, at a time therefore when the potassium is sufficient to lower distinctly the efficiency of the heart contractions. It is not possible to state precisely at what concentration the depressing influence of the potassium on the vagus action begins, since different hearts react differently in this respect. As stated above, an increase to 0.07 per cent is associated usually with a distinct augmentation of the inhibitory influence of the vagus while at 0.09 to 0.10 per cent the contrary effect may be noticeable. So long as the increase of the potassium constituent exercises no obviously weakening effect upon the heart it serves to increase its responsiveness to the action of vagus impulses. The depressing effect of large doses of potassium chloride upon vagus inhibition is similar to that shown by muscarin. At one stage in the action of this drug, when under its influence the beats of the heart are slowed, stimulation of the vagus has no effect upon the heart.<sup>1</sup>

## II. EFFECT OF REMOVAL OF THE POTASSIUM SALTS FROM THE CIRCULATING LIQUID.

The fact that an increase in the amount of diffusible potassium compounds in the heart favors inhibition, suggested naturally the possibility that, by supplying the heart with a circulating medium free

<sup>1</sup> WEINZWEIG: *Archiv für Physiologie*, 1882, p. 532.



from potassium, the amount of this element present in the heart in diffusible form might be reduced to such an extent as to suspend entirely the inhibitory action of the vagus. In the series of experiments carried out to test this idea both frogs and terrapins were used. The heart was isolated and kept beating by an artificial circulation of a standard Ringer's mixture. In the frog's heart it was found necessary, in order to obtain forceful efficient contractions, to add to the Ringer's mixture some sodium carbonate (0.003 per cent). The sensitiveness of the vagus was determined by finding the strength of induced current which sufficed to stop completely the contractions of the auricles or the ventricle. In the frog the combined vago-sympathetic trunk was stimulated, since stimulation of the intracranial portion of the vagus did not prove to be feasible under the conditions of the experiment. After ascertaining the sensitiveness of the heart to vagus stimulation the circulating liquid was changed from the neutral or alkaline standard Ringer's solution to one consisting of sodium chloride alone (0.7 per cent), or sodium chloride (0.7 per cent) plus calcium chloride (0.023 per cent). The effects of these two solutions may be considered separately.

**Action of the sodium chloride (0.7 per cent).**—When the heart is irrigated with an isotonic solution of sodium chloride the contractions of all chambers quickly become feeble, while the rate may be accelerated. All parts of the heart dilate greatly owing to loss of tone. The systole of the ventricle is especially incomplete, the contractions soon becoming restricted almost entirely to small movements at the base. While in this condition stimulation of the vagus may be entirely without effect upon the contractions of either the auricles or the ventricle. A similar observation has been reported recently by Wybau.<sup>1</sup> This author states that the loss of control of the vagus over the ventricle becomes apparent only after the ventricle has assumed an independent rhythm, and that to obtain this end, by his method, required an irrigation of the ventricle during some hours. In his method, however, the ventricle only was irrigated with the saline, by a perfusion cannula, the auricle and sinus receiving the animal's own venous blood. By the method that I used, the whole heart was irrigated with the solution of sodium chloride, and the condition of independence of vagus control developed within a few minutes, the auricle as well as the ventricle being entirely unaffected by vagus stimulation.

<sup>1</sup> WYBAU: Archives internationales de physiologie, 1904, ii, 198.

A similar experiment was described long ago by Schiff,<sup>1</sup> and later by Löwit,<sup>2</sup> who obtained the same results by the use of solutions of sodium sulphate and sodium carbonate. This latter observer used, however, concentrated solutions of these salts applied directly to the heart or injected into the lymph sinuses. If the exposure to the saline solution is not maintained for too long a period, a return to the standard Ringer's solution is followed by a reappearance of vagus inhibition, but if the irrigation with the sodium chloride solution is continued for a longer period, it may happen that a return to the Ringer's mixture, although followed by a revival of regular forceful beats in all chambers, is not accompanied by a return of vagus control. It seems probable, therefore, that the effect of solutions of sodium chloride on vagus inhibition is due in part to some injury to the endings of the vagus nerve in the heart, in addition to a possible direct action upon the heart muscle owing to a diffusion out of its potassium and calcium compounds. Wybau's generalization that the loss of vagus control is associated with an assumption of independent rhythmicity by the ventricle is not borne out by my experiments. The phenomenon appeared with this circulating medium when the heart beats, although feeble, showed a regular auriculo-ventricular sequence. For the sake of completeness it should be added that in some cases with the frog's heart, in which the irrigation was effected with a solution of sodium chloride made alkaline by sodium carbonate, the vagus inhibition was retained for a longer period, and in these cases the heart beats did not show the feeble incomplete contractions observed when the neutral saline was employed.

**Action of a mixture of sodium chloride and calcium chloride (Ringer's mixture without potassium).**— With this circulating liquid the heart continues to give vigorous beats. The first effect of the solution is usually to throw the heart into a condition of increased tone, this effect being especially marked upon the auricles which show a decided diminution in volume. The heart may also in the beginning exhibit quicker and more forcible contractions, but later the ventricular systoles become more prolonged, and a condition resembling partial or complete heart-block makes its appearance.

The ventricle responds to every second or third auricular contraction and finally may become entirely independent of the auricles,

<sup>1</sup> SCHIFF: Archives des sciences physiques et naturelles, 1877-78; also Recueil des mémoires physiologiques, 1894, i, p. 652.

<sup>2</sup> LÖWIT: Archiv für die gesammte Physiologie, 1881, xxv, 466.

or fall into a condition of irregular and infrequent beats. In the terrapin's heart the sodium calcium mixture sometimes causes the ventricle to assume an independent rhythm very abruptly, the beats changing at once from the rapid auricular rate to the slower ventricular rhythm and changing back again promptly when a Ringer's mixture containing potassium is passed into the heart. Whatever may have been the final effect upon the ventricle, and this varied in different animals, the result upon the auricles was uniform. After a preliminary augmentation of tone this portion of the heart continued to beat with regularity throughout the duration of the experiments lasting from a few minutes to one or two hours.

The effect of this sodium calcium mixture upon vagus inhibition of the ventricle of the terrapin's heart was clear and unmistakable. Stimulation of the vagus was entirely without effect upon the ventricular beats. The auricles might be inhibited and come to a complete standstill, but the ventricle continued to beat, with or without a brief preliminary pause, at its own slower independent rhythm. The simplest explanation of this uniform result is found in the suggestion made by Gaskell. According to this observer the vagus in the terrapin has no direct control over the ventricle. Ordinarily the ventricle stops when the vagus is stimulated because the auricles are inhibited, and consequently it receives no impulse from these chambers. Normal blood is not capable of stimulating the ventricle to an independent rhythm. When, however, the heart is irrigated with the sodium calcium mixture, the inhibition of the auricle while freeing the ventricle from auricular control, allows the potassium free circulating liquid to set up an independent ventricular rhythm. This explanation is supported by the results of experiments upon frogs. In the frog there is no doubt that the inhibitory fibres of the vagus control directly both the contractions of the ventricle and of the auricle. In accordance with this fact it was found that in this animal the auricle and ventricle behaved alike. If the sodium calcium mixture abolished vagus inhibition of the ventricle, it had the same effect on the auricle.

So far as the terrapin's heart is concerned, the true relation of the potassium compounds to the property of inhibition through the vagus is to be found in the reaction of the auricles, the part of the heart which is undoubtedly under the direct control of these nerves. The results actually obtained in the present series of experiments were not entirely uniform. In some of the experiments the contraction of

the left auricle was registered by attaching its tip to a lever, while in other cases in order to maintain entirely normal conditions both auricles were fully exposed, by cutting the arterial branches close to the ventricle, and were then simply observed. In a series of seven experiments upon terrapins made in the spring of 1905, it was found in all cases that the entire heart was completely inhibited with weak stimuli when irrigated with the standard Ringer's mixture (KCl 0.03 per cent). If the irrigating solution was changed to the sodium calcium mixture then, after a shorter or longer period, stimulation of the vagus with either weak or strong stimuli failed to arrest the auricle, or if any inhibitory effect was obtained, it was confined to the right auricle or was characterized by a long latent period, that is, the inhibition developed very gradually. On changing back to the standard Ringer's mixture complete and prompt inhibition of all chambers was readily obtained. In two similar experiments on the terrapin made in the fall of 1905, one heart when irrigated with the sodium calcium mixture gave complete inhibition of the auricles upon stimulation of the vagus, although the ventricle continued to beat. The stoppage of the auricles was, however, preceded by a long latent period (10 beats), the chamber during this period exhibiting a gradual dilatation from loss of tone. In the other heart, while on the sodium calcium mixture, stimulation of the vagus served only to weaken the beats of the auricles without bringing them completely to rest, the effect upon the right auricle being more distinct than that upon the left. In both cases a return to the potassium Ringer was followed by prompt complete inhibition of all chambers when the vagus was stimulated.

In a series of eight experiments upon frogs, also made in the fall (1905), less decided results were obtained. In four of these experiments the heart continued to give complete inhibition of all chambers when irrigated with the sodium calcium mixture, the inhibition being as prompt and complete as under normal conditions. In two of the other four experiments the heart showed at first complete inhibition of all chambers for a brief period, then the ventricle began to beat and was followed in order by auricle and sinus. That is, as long as the vagus was stimulated the heart showed a reversed rhythm, ventricle-auricle-sinus, which persisted for a short period after cessation of the stimulus and then suddenly changed to the normal sequence. A satisfactory explanation of this result is to be found in the fact that both the inhibitory and accelerator fibres were being stimulated,

and the action of the latter was favored while that of the former was depressed by the lack of potassium salts in the circulating mixture. Since the accelerator is known to influence the ventricle directly it may be that its action caused this chamber to beat first, and the ventricular systole was then followed in sequence by auricle and sinus, as happens usually when the ventricle is made to beat during vagus inhibition. In the other two experiments only the auricles beat well upon the sodium calcium mixture, and in the numerous stimulations made it was found that on the sodium calcium mixture the auricles could not be inhibited completely. If there was any cessation, it was only momentary and was followed at once by an escape. Return to a potassium mixture was accompanied by a complete and long-lasting inhibition whenever the vagus (left) was stimulated. It was observed, however, that after exposure to the sodium calcium mixture a return to the standard Ringer with 0.03 per cent potassium chloride gave no inhibition; to obtain this result, it was now necessary to raise the percentage to 0.05 or 0.07. It is evident from these experiments that the *direct inhibitory influence of the vagus upon the heart may be lost entirely when the circulating liquid contains no potassium salts, and that in such cases this influence is restored promptly by the addition of these salts in physiological amounts.*

The fact that in some cases, in the frog's heart, the vagus nerve inhibited all chambers of the heart, although potassium salts were not present in the circulating liquid, may be explained by assuming that in such cases the production of new potassium compounds in diffusible form took place in the tissue so rapidly that they could not be removed by diffusion with the circulating liquid. It is possible that a seasonal variation may enter as a factor in this regard. When one circulates a sodium calcium mixture through the heart, it is not probable that all the diffusible potassium compounds are thereby completely removed from the heart tissue. What is present in the tissue lymph may diffuse out promptly, but it is highly probable that more will be formed by dissociation of the relatively abundant supply of potassium material within the muscle. This consideration may explain the fact that in the different hearts examined different degrees of effects were obtained, varying from a complete loss of inhibition to a condition in which the action of the vagus was apparently unaffected. The significant cases certainly are those in which removal of potassium was followed by loss of power of inhibition, and in which this power was restored by the addition of potassium salts.



So far as our facts go at present, they warrant the probable conclusion that in the absence of diffusible potassium compounds in the substance of the heart muscle rhythmic automatic contractions may still persist, but the possibility of inhibition through the vagus nerve is removed.

### III. EFFECT OF INCREASING THE AMOUNT OF CALCIUM SALT IN THE CIRCULATING LIQUID.

This series of experiments was carried out entirely upon terrapins. Beginning with the standard Ringer's mixture containing 0.023 per cent of calcium chloride, the calcium percentage was increased to 0.138 per cent through five stages (0.046, 0.069, 0.092, 0.115, and 0.138 per cent). The results from six experiments of this character were quite uniform. The end sought for was to determine the minimal stimulus applied to the vagus which was necessary at each concentration to cause complete inhibition of the auricles or the ventricle. So far as the ventricles were concerned it was found that as the calcium contents of the circulating liquid were increased inhibition became more difficult, that is, necessitated a stronger stimulus. At a certain concentration (0.10 per cent), which varied somewhat for the different hearts, complete inhibition failed entirely with the strongest stimuli, and in all probability stimulation of the vagus failed to have any effect upon the ventricle. That is to say, stimulation of the vagus under these conditions might be followed by a slower rate of the ventricular beats, while the auricle stopped completely in diastole. The slower rhythm of the ventricle was due probably, however, not to a direct inhibition, but to the fact that on stoppage of the auricles it began beating at once with its own slower rhythm on account of the higher concentration in calcium salts, a condition which, as is well known, develops an independent automatic rhythmicity in the ventricle of this animal. On the basis of these experiments we may conclude, therefore, that *as soon as the concentration of calcium salts in the circulating liquid is sufficient to develop an independent beat in the ventricle, vagus stimulation ceases to have any effect upon this part of the heart.*

The conclusion applies only to those ventricles upon which the vagus has little or no direct influence. Gaskell's statement that in the reptilian heart the vagus has no direct effect upon the ventricle and can influence its beat only through an effect upon the auricle is



in accord with all of my observations, except perhaps one. In one experiment, while irrigating the heart with the standard Ringer's mixture, both auricles and ventricle were inhibited completely by stimulating the vagus with a current strength of 75. The same result was obtained with a concentration in calcium chloride of 0.046 per cent, but at a concentration of 0.069 per cent the auricle alone was completely inhibited by a strength of 75, while the ventricle showed only a slowing of rate. On increasing the strength of stimulus, however, to 300 the ventricle as well as the auricles was brought to a complete stop. With higher concentrations of calcium chloride the ventricle could not be inhibited completely with any strength of stimulus, but assumed simply its slower independent rhythm, as described above. This experiment would indicate that the vagus may have, in some individuals at least, some direct connection with the ventricular muscle, although its influence upon this part of the heart is obviously less complete than in the case of the auricles.

With regard to the auricles quite a different result was obtained. With all concentrations of calcium chloride used the auricles were completely inhibited upon stimulation of the vagus, and, indeed, the minimal stimulus necessary to produce this effect remained unchanged throughout. We may conclude therefore that *so long as the concentration of the circulating liquid in potassium and sodium salts remains within normal limits, variations in the calcium salts (0.023 per cent to 0.138 per cent calcium chloride) have no influence upon the vagus inhibition of the auricles.*

#### THEORETICAL.

The very great and indeed essential importance of the inorganic salts to the normal properties of heart-muscle has been abundantly demonstrated within recent years. The facts that have accumulated force us to believe that the normal functions of the protoplasmic material of the heart depend upon the presence of a certain number of these salts, particularly those of sodium, potassium, and calcium, in certain definite proportions, and we cannot escape the conviction that these salts or their ions form compounds with the complex organic substances in the heart-tissue, compounds which perhaps may be regarded as addition products, and which are capable of dissociation.

From the experiments given above it would appear that vagus inhi-

hibition of the heart is not possible when the tissue-lymph is depleted of its diffusible potassium salts, under conditions which ensure the retention of the other salts essential to normal activity. This fact, or probable fact, may be understood if it is assumed that the substance whose dissociation and oxidation yields the energy of contraction forms with the potassium salts a relatively stable compound, or at least has its property of dissociation prevented in some way by the presence of potassium salts, and that this action of the potassium constitutes the initial cause of inhibition. The analogy between the inhibition produced by excess of potassium salts in the circulating liquid and that caused by the stimulation of the vagus nerve is most striking,<sup>1</sup> and now that it appears that potassium salts in excess within certain limits favor vagus inhibition, while their absence or serious diminution in quantity retards or altogether prevents the inhibitory action of the vagus, it seems justifiable to assume as a provisional hypothesis that vagus inhibition takes place because the nerve impulses effect a sudden increase in the amount of diffusible potassium compounds in the heart-substance. We know from the work of Macallum<sup>2</sup> and others that the heart contains an abundant supply of potassium. This material is contained in organic combination, but probably as a compound that is capable of dissociation with the liberation of potassium. The possibility of the existence of such compounds and of their dissociation is indicated by the recent microchemical work of MacDonald<sup>3</sup> upon the nerve fibre. It is quite conceivable therefore that the action of the vagus impulses consists primarily in augmenting such a dissociation and thus setting free an inhibitory substance, namely potassium, the presence of which, in excess of its usual amount, prevents the dissociation of the so-called contractile substance. Among the theories advanced to explain the

<sup>1</sup> It is sometimes stated that arrest of the heart by potassium differs from vagus inhibition in the fact that in the latter condition the heart is still responsive to external stimuli, while in the former it is not. MARTIN has shown, however, that with a dose of potassium chloride sufficient to stop the ventricle, this chamber may still be irritable to mechanical stimuli. On the other hand, LANGENDORFF states that muscarine applied directly to the heart destroys its irritability toward external stimuli, while ECKHARD, SCHIFF, and GASKELL have shown that stimulation of the vagus may also weaken or suspend the irritability of the heart.

<sup>2</sup> MACALLUM: *Journal of physiology*, 1905, xxxii. p 95.

<sup>3</sup> MACDONALD: *Journal of physiology*, 1905, xxxii. *Proceedings of the Physiological Society*. also *Proceedings of the Royal Society*, London, 1905, B, lxxvi, p. 322.

phenomenon of inhibition, that proposed by Gaskell<sup>1</sup> and Hering has found most favor among physiologists, or at least is most frequently referred to. According to Gaskell the inhibitory fibres of the vagus constitute an anabolic nerve to the heart-muscle, that is, its impulses cause an increase in the anabolic or assimilatory processes within the muscle. In favor of this view it has been urged that as an after-effect of vagus stimulation there is an increase in the force of the heart-beat and in the conductivity of the tissue, and, moreover, that during vagus inhibition the part of the heart affected shows an increased positive potential with reference to an injured area. In regard to the increase in the force of the heart beat observed after vagus inhibition, it must be remembered that this phenomenon is exhibited by the ventricle of the terrapin, and yet according to Gaskell this part of the heart in this animal is not actually inhibited, it simply comes to rest because the auricle is inhibited. It would seem from this that an increase in the force of contraction may follow a condition of rest, that is, absence of catabolism, and cannot therefore be cited as a proof of an increase in anabolism due to external influences.

As a matter of fact, we recognize in the inhibited heart muscle only a condition of suspended activity, a prolonged diastole, during which there is no evidence of disassimilation or physiological oxidations. It is somewhat difficult to conceive in the first place how a nerve impulse can lead to an increase in the assimilatory processes, but, granting that it may, it is not clear why such a condition should be accompanied by a cessation of catabolism. In other organs, such as the glands, an increased catabolism during activity goes hand in hand with a greater anabolism, and conditions, such for instance as a higher temperature, which may augment the anabolic activity of the living substance increase also the extent of the catabolic changes. This effect we should expect on the usual assumption that anabolism is essentially a synthetic process, whereby more complex and more unstable compounds are formed. The heart, however, may be inhibited in the cold-blooded animals during many hours. To suppose that during such long periods anabolic processes are proceeding continuously, to the exclusion of all catabolic changes, is to imagine a process of synthesis or polymerization of a practically indefinite extent. With regard to the argument that the muscle during inhibition shows an increased positive potential and therefore must be in a state of anabolism,

<sup>1</sup> GASKELL: Philosophical transactions, London, 1881, clxxiii, p. 1029.

because during catabolism there is the opposite condition of negative potential, it is of course easy to point out that the logic of the reasoning is not entirely sound. From the standpoint of physical chemistry the acquisition of an electrical charge implies a process of dissociation and formation of ions, and if anabolism consists, as is usually assumed, in the synthetic combination of certain unsaturated groupings, it should result in the production of electrically neutral compounds. According to the theory proposed in this paper, the vagus impulses, like those of motor nerves, set up a dissociation with the liberation of electrolytes in an ionized condition. It is assumed that the electrolyte in question is a potassium compound which acts as an inhibitory substance toward the energy yielding material. Upon this assumption we may understand the production of a positive potential, if, with Oker-Blom, we suppose that the kations of the potassium compounds diffuse more rapidly than the anions. This latter observer, in fact, has shown<sup>1</sup> that when one end of a fresh, uninjured sartorius muscle is treated with water or dilute solutions of potassium chloride, the end so treated becomes electro-positive to other parts of the muscle. He explains this result on the theory that a dissociation results with the liberation of electrolytes, and a more rapid migration of the positively charged ions through the surrounding sheaths. The work of Langley<sup>2</sup> and of Erlanger<sup>3</sup> make it highly probable that there is no qualitative difference between the impulses transmitted by motor and inhibitory fibres, since a motor nerve, when led down an inhibitory path, continues to give inhibition. In the case of the heart the difference in action between the inhibitory and accelerator fibres must be referred in the long run to the fact that they end in different structures or substances. Histological work, it is true, does not support such a view, and on the chemical side our knowledge of the heart metabolism is far too meagre to justify any detailed hypothesis, but the physiological facts known regarding cardiac inhibition are in accord with the idea that the vagus fibres end in what might be designated as an inhibitory substance, which, under the influence of its impulses, is dissociated with the liberation of potassium compounds to which the phenomenon of inhibition is directly due.

<sup>1</sup> OKER-BLOM: *Archiv für die gesammte Physiologie*, 1901, lxxxiv, p. 191.

<sup>2</sup> LANGLEY: *Journal of Physiology*, 1904, xxx, p. 439.

<sup>3</sup> ERLANGER: *This journal*, 1905, xiii, p. 372.

## CONCLUSIONS.

In a circulating medium containing only sodium, potassium, and calcium chlorides

1. An increase in the amount of the potassium salt augments the sensitiveness of the heart to vagus inhibition, until the amount of the potassium becomes sufficiently large to cause itself marked inhibitory effects. Under the last condition the effect of vagus inhibition is greatly lessened.

2. Complete lack of potassium salts is attended by a diminution in, or entire loss of, vagus control of the different chambers of the heart.

3. A solution containing only sodium chloride (0.7 per cent) causes loss of vagus control of the heart-beats.

4. An increase in the calcium salts (0.023 to 0.138 per cent) has no effect upon vagus inhibition of the auricles. The same change is followed by diminution and finally entire loss of vagus action on the ventricle (terrapin), owing probably to the development of an independent ventricular rhythm.

5. The results of the experiments are interpreted to indicate that inhibition of the heart depends upon the presence of diffusible potassium compounds in the heart tissue, and that the vagus impulses act indirectly by increasing the amount of potassium compounds of this character.



## A DETERMINATION OF THE ERRORS OF ECCENTRICITY AND COLLIMATION IN THE HUMAN EYE.

BY FREDERICK E. BEACH.

[From the Department of Physics in the Sheffield Scientific School of Yale University.]

IN a recently published paper<sup>1</sup> Professor Hastings has pointed out that the method employed by Helmholtz for determining whether the optical surfaces in the human eye are accurately centred was inadequate to resolve the question, and incidentally attention was called to the fact that even accepting Helmholtz's conclusion that the eye is not truly centred, no indication was given either of the amount or direction of this departure, nor any method for determining whether this error was one of collimation or of eccentricity.

It is the aim of this paper to show the answers to these questions, which, so far as the writer has been able to determine, have remained open up to the present time. An understanding of the quantities involved in the discussion may be obtained from a consideration of Fig. 1, which represents a horizontal section of the right eye seen from above. Thus,  $VC$  represents the surface of the cornea. Since the curvature of this surface diminishes toward the edges, the axis of symmetry  $VK$  may be called the corneal axis. The vertex is designated by  $V$ , and  $K$  is the corresponding centre of curvature.  $A$  represents the anterior and  $P$  the posterior surface of the lens;  $ML$  is the axis of the lens and  $L$  its centre of figure.  $FL$  is the line of most distinct vision, and since the direction of any fixation point,  $F$ , is precise and easily determined, this line has been chosen as an axis of reference.

The axis of the cornea always lies on the temporal side of the axis of vision, by an amount which varies notably in different eyes.<sup>2</sup> The average value of this angle, commonly designated by  $\alpha$ , is not far

<sup>1</sup> This journal, 1905, xiii, p. 304.

<sup>2</sup> HASTINGS: American journal of science, 1905, xix, p. 310.



from  $4^\circ$ . The eyes studied in the present investigation had had this constant determined for the paper just quoted.

It is now further assumed that the axis of the lens has been rotated about the centre  $L$ , an amount  $\Delta u$  (the collimation error), the rotation from  $F$  toward  $M$  being counted positive, and also displaced laterally an amount  $\Delta y$  (the error of eccentricity), measured posi-

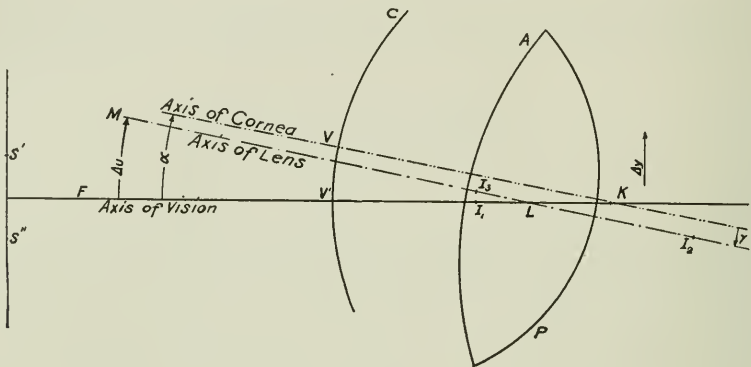


FIGURE 1.

tively in a direction from  $F$  toward  $M$ . To determine the value of these errors two different methods were employed.

**First method.**—In the first method I have availed myself of the following calculations made by Professor Hastings on the schematic eye. Let  $I_1$  denote the image formed by reflection at the surface  $C$ ;  $I_2$ , the image formed by refraction at  $C$ , reflection at  $A$ , and refraction again at  $C$ ; and  $I_3$ , that formed by successive refractions at  $C$  and  $A$ , reflection at  $P$ , and finally refractions again at  $A$  and  $C$ . If, now, the axis of the cornea be assumed coincident with the axis of the lens, and a luminous point be placed at a distance of 100 cm. in front of the cornea on this common axis, the distances of these images from the vertex of the cornea as calculated from the constants of the schematic eye were found to be,—

$$x_1 = 0.3899 \text{ cm.}$$

$$x_2 = 1.051 \text{ cm.}$$

$$x_3 = 0.3942 \text{ cm.}$$

If the axis of the lens be rotated an amount  $\Delta u$  about  $L$ , the corresponding displacement of  $I_2$  from the axis will be  $-1.448 \text{ cm. } \Delta u$ , and that of  $I_3$ ,  $0.413 \text{ cm. } \Delta u$ . Likewise, if the axis of the lens be displaced laterally an amount  $\Delta y$ , the corresponding movement of  $I_2$

will be  $1.764 \Delta y$ , and that of  $I_3$   $1.058 \Delta y$ . Hence, if the ordinates  $y_2$  and  $y_3$  can be found, the errors of collimation and eccentricity can be calculated from the equations

$$\begin{aligned} -1.45 \text{ cm. } \Delta u + 1.76 \Delta y &= y_2 \\ 0.413 \text{ cm. } \Delta u + 1.06 \Delta y &= y_3 \end{aligned}$$

The experimental method used for determining these ordinates was the following: The eye to be examined was directed toward a tele-

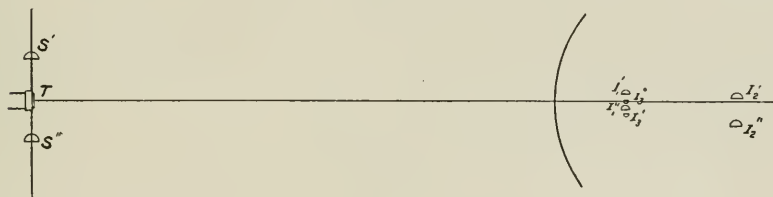


FIGURE 2.

scope  $T$ , Fig. 2, about a metre distant. Lights placed at equal distances, 10 cm. on either side, gave rise to a series of six images as shown. The brightest, or corneal images, were used as points of reference, and the positions of the others with respect to them were measured by the aid of a micrometer eyepiece. The point half-way between each pair was assumed to be the position which would have been occupied by the images if the sources had been made to coalesce. A difficulty which presents itself at once in the actual measurements is that the light of one of the hazy images is commonly cut off by the iris, even with the greatest dilation of the pupil obtainable in a dark room. In this case the subject was asked to direct his vision to a movable target,  $F$ , which was shifted by the observer until the hitherto invisible image  $I_2''$  came into the field. Its position and the new place of  $I_2'$ , which remains visible during the motion, is then determined. The original position of  $I_2''$  can now be calculated on the assumption that during this rotation of the eye the movement of  $I_2''$  has been the same as that of  $I_2'$ , which is known. This assumption may be objected to, on the ground that since  $I_2''$  is farther from the axis of the figure than  $I_2'$ , its movement must differ somewhat from that of the latter. It may be noted, on the other hand, that great precision of measurement cannot be secured without a study of all the constants of each particular eye, and all that is sought is to establish the existence of systematic

errors of collimation or eccentricity. However, in each case as a check on the preceding assumption the position of  $I_2''$  was found independently from the relative magnifications of the images which are in the ratios, —

$$I_1 : I_2 : I_3 = .1 : 1.908 : -0.7297$$

It is obvious that the separation in the corresponding pairs of images, in Fig. 2, should agree very nearly with these ratios. The position of  $I_2''$  accepted for the final calculation was the mean of the positions found in these two ways. It may be remarked that the effect of this latter correction has been to give values to  $\Delta u$  in the table which are probably slightly less than their true amounts. This table

TABLE I.

	RIGHT EYE.						LEFT EYE.					
	$\Delta u$ Temp.	$\alpha$ Temp.	$\Delta y$ Temp.	$\Delta y$ Nas.	$\gamma$ Temp.	$\gamma$ Nas.	$\Delta u$ Temp.	$\alpha$ Temp.	$\Delta y$ Temp.	$\Delta y$ Nas.	$\gamma$ Temp.	$\gamma$ Nas.
H. R. . .	9.80°	7.76°	mm. ....	mm. 0.004	2.04°	....	9.05°	7.05°	mm. 0.006	mm. ....	2.00°	....
E. L. . .	4.90°	6.51°	0.215	....	....	1.61°	3.84°	6.56°	0.154	....	....	2.72°
L. W. . .	6.30°	5.07°	0.135	....	1.23°	....	4.81°	4.35°	0.088	....	0.46°	....
C. P. . .	7.63°	4.34°	....	0.118	3.29°	....	7.67°	5.05°	0.007	....	2.62°	....
H. D. . .	5.47°	3.68°	0.071	....	1.79°	....	5.63°	4.87°	....	0.043	0.76°	....
W. M. . .	4.68°	3.45°	....	0.116	1.23°	....	3.21°	1.25°	....	0.152	1.96°	....
J. M. . .	3.82°	3.42°	0.174	....	0.40°	....	3.95°	3.26°	0.241	....	0.69°	....
C. H. . .	2.90°	3.37°	....	0.060	....	0.47°	2.53°	1.49°	0.007	....	0.64°	....
O. L. . .	7.28°	3.14°	0.003	....	4.14°	....	6.78°	2.77°	0.015	....	4.01°	....
S. D. . .	4.47°	2.64°	....	0.006	1.83°	....	4.78°	3.58°	....	0.041	1.20°	....
A. E. . .	3.87°	1.68°	0.124	....	2.19°	....	3.32°	1.64°	0.142	....	1.68°	....
L. M. . .	3.39°	1.58°	....	0.018	1.81°	....	3.12°	1.51°	0.042	....	1.61°	....
F. B. . .	3.89°	1.55°	....	0.066	2.34°	....	3.06°	2.41°	....	0.128	0.65°	....
S. P. . .	3.86°	....	0.128	....	....	....	4.11°	....	0.125	....	....	....
Mean . .	5.17°	3.71°	0.121	0.055	1.20°		4.71°	3.52°	0.083	0.091	1.55°	

exhibits the results found in twenty-eight eyes. The letter  $\gamma$  has been introduced to designate the angle between the axis of the cornea and the axis of the lens.

**Second method.** — The observations for the preceding calculations may also be made to yield the errors of collimation and eccentricity in a simple manner from a consideration of the purely geometric relations involved. Thus, let Fig. 3 represent the eye with the fixation

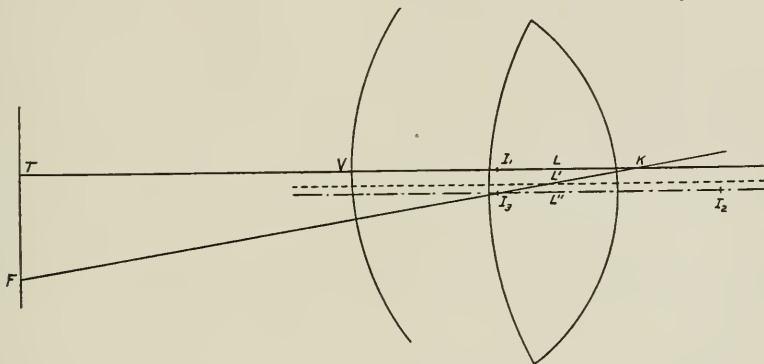


FIGURE 3.

point  $F$  so chosen that the axis of the lens is parallel to  $KT$ , on which the light is placed, a long distance in front. This will be accomplished when  $I_2$  and  $I_3$  appear to fall together, and the required error of collimation will be  $TKF$ . The apparent coincidence of  $I_2$  and  $I_3$  as seen from the telescope was not carried out experimentally, but was deduced from the observations which gave the change of place of each image per centimetre change of the fixation point.

This rate, which may be represented mathematically by  $\frac{\delta I}{\delta F}$ , differed somewhat in different eyes, but it was assumed that the mean found from a few eyes would serve as a fair check on the results obtained by the first method.  $I_2$  and  $I_3$  having been made to coincide, and the lateral separation of  $I_1$  and  $I_3$ , Fig. 3, also being known, the error of eccentricity may be found from it as follows: Suppose first that there is no lateral displacement of the axis of the lens and that the fixation point corresponds to  $T$ . The centre of the lens would then be at  $L$ . If the fixation point be now changed to  $F$ , the centre of the lens will move to  $L'$ , a distance  $LL' = L'K \sin LKL'$ . In the schematic

eye the distance  $L'K$  from the centre of the lens to the centre of curvature of the cornea is 0.24 cm. As  $LKL'$  is a known quantity,  $LL'$  may be readily found. The difference between the ordinate  $I_1I_3$  and  $LL'$  is obviously the eccentricity,  $L'L''$ , or  $\Delta y$ .

**Comparison of the two methods.**—In order to determine whether the values found for the eccentricity had a real existence or were to be ascribed to the roughness of the observations, the eighteen eyes for which this quantity appeared to be the largest were selected and the numbers recalculated by the second method. The values of  $\frac{\delta I_2}{\delta F}$  and  $\frac{\delta I_3}{\delta F}$  had been fully observed on only six eyes, so that a somewhat uncertain average of these coefficients had to be used instead of the value proper to each eye. The results were as follows: The mean value of  $\Delta u$  by the first method was  $4.97^\circ$ , and by the second  $4.90^\circ$ , or a difference in the means of  $0.07^\circ$ . The mean of the individual differences by the two methods, neglecting the sign, was  $0.24^\circ$ .

The mean value of  $\Delta y$ , also taken without regard to sign, by the first method was 0.101 mm., and by the second 0.133 mm., or a difference between the means of 0.032 mm. The mean of the individual differences in this case was found to be 0.076 mm. Although these differences for the error of eccentricity are nearly of the order of magnitude of the quantities measured, it so happened that the direction of the lateral displacement of the axis was found to be the same by both methods in seventeen out of the eighteen cases.

#### SUMMARY OF RESULTS.

1. The axis of the lens is inclined to the axis of vision, and the point where the axis of the lens intersects the cornea lies systematically on the temporal side of the point where the axis of vision intersects this surface. Mean error of collimation in twenty-eight eyes,  $4.9^\circ$ .

2. In the great majority of eyes, say 85 per cent, the point of intersection of the axis of the lens with the cornea lies on the temporal side of the vertex of the cornea. Mean value of  $\gamma$ ,  $1.4^\circ$ .

3. The centre of the lens is not displaced from the axis of vision by an appreciable amount; that is to say, the error of eccentricity is insignificant. Mean value, 0.086 mm. In seventeen eyes the dis-

placement was toward the temporal side ; mean value, 0.10 mm. In eleven eyes the displacement was toward the nasal side ; mean value, 0.07 mm.

4. The second method of investigation probably gives the more accurate values, since it is self-contained and takes account of the peculiarities of each eye.





## A STUDY OF THE ABSORPTION AND CONSUMPTION OF OXYGEN IN HEART TISSUE.

By E. G. MARTIN.

[From the Physiological Laboratory of Purdue University, Lafayette, Ind.]

IT has been a matter of discussion from time to time how far the beat of the cold-blooded ventricle, immersed in physiological saline, is influenced by the amount of oxygen in solution in the bath. The general impression seems to have been that the moderate demands of cold-blooded heart tissue for oxygen are fully met by the amount normally contained in the solution. Howell,<sup>1</sup> commenting upon this subject says, "Fortunately, in experiments upon the effect of solutions of the inorganic salts, this factor [the oxygen supply] does not have to be considered, since the aqueous solutions used hold sufficient oxygen in solution for the tissues of the cold-blooded animal." Lingle,<sup>2</sup> on the other hand, has taken the view that the tissue is influenced markedly by the amount of oxygen in the bath. He suggests that the "sodium chloride arrest" may be due to a mild asphyxia, resulting from insufficient oxygen. He bases this idea chiefly upon the fact that thorough oxygenation of a sodium chloride solution in which a strip has ceased to beat results in a marked renewal of activity. Those who take the view expressed by Howell are led to it by a number of facts, chief among which are these: Strips immersed in sodium calcium mixtures or Ringer's solutions may, under proper conditions, continue active for very long periods without any special precautions being taken to insure an oxygen supply. Addition of a small per cent of calcium chloride or an alkali to a sodium chloride solution in which exhaustion has occurred may bring about an excellent recovery. Exhaustion comes on as quickly in a large volume of sodium chloride solution as in a small one, although in the latter case the total amount of available oxygen is certainly much less than in the former.

<sup>1</sup> HOWELL: This journal, 1901, vi, p. 193.

<sup>2</sup> LINGLE: *Ibid.*, 1902, viii, p. 98.

It has seemed to the author worth while to make a more careful study than has yet been made of the influence of oxygen upon the activity of ventricular strips, so that this factor may be taken into account when conclusions are being drawn from experiments in which it might enter. The method of experimentation was essentially the same as that usual for investigations upon heart strips. For holding the bathing solutions broad tubes were used, having a capacity of 25 c.c. each. In the top of each tube was fitted a tight stopper, pierced with a small hole for the passage of the thread leading to the recording lever. The purpose of this stopper was to reduce as much as possible the diffusion of gases between the solution and the air. The apparatus was so arranged that the solutions could be thoroughly saturated with any desired gas, by causing it to bubble through them. Every experiment was carefully controlled, and the usual precautions were taken to insure purity of the solutions. Turtles' hearts were used throughout.

#### DEFICIENCY OF OXYGEN.

Howell<sup>1</sup> studied the series of contractions given by a strip in sodium chloride solution free from oxygen. He found that the effect of the absence of oxygen in the surrounding medium was to diminish the extent of contraction, but in other respects was not at all marked. He calls attention, however, to the fact that in such an experiment the strip contains at the beginning a considerable supply of oxygen which cannot be removed, and which is doubtless available for the use of the tissue.

The author performed a number of experiments in which the influence of oxygen-free baths of various kinds was studied. The method of freeing a solution of oxygen was to boil it and pour it into a tube, which was quickly stoppered and placed under water to cool. When the strip to be tested had been hung in position in its tube, nitrogen gas, prepared from ammonium nitrite, was passed freely through the tube to sweep out the atmospheric oxygen. The oxygen-free solution was then passed in until the strip was immersed. Usually a gentle stream of nitrogen was kept bubbling through the solution so that a layer of that gas might always cover its surface and prevent any inward diffusion of atmospheric oxygen. The use of  $\frac{2}{3}$  sodium chloride as a bath gave the following results: The latent

<sup>1</sup> HOWELL: *Loc. cit.*, p. 193.

period in the oxygen-free bath was shortened — for eight experiments the average values were: oxygen-free solution, thirteen minutes; ordinary solution, twenty-three minutes; the length of the series of contractions was slightly diminished — for six experiments the averages were: oxygen-free solution, thirty-nine minutes; ordinary solution, fifty-one minutes. In none of these experiments was there a marked diminution of vigor such as Howell observed in a similar case; a preliminary bath of a calcium-containing solution which, as the author pointed out in a previous paper,<sup>1</sup> is ordinarily very effective in shortening the latent period, was apparently without effect in the absence of oxygen; after exhaustion in an oxygen-free bath, excellent and long-continued recovery could be obtained by thorough oxygenation of the solution, or by transferring the strip to a moist chamber; the same procedure also resulted in an equally good recovery after exhaustion in an ordinary bath; the addition of a calcium salt to the solution in which exhaustion has taken place, a procedure which results in an excellent recovery ordinarily, was without effect when applied to an oxygen-free solution; the addition of an alkali, on the other hand, gave as good a recovery in the absence of oxygen as in an ordinary solution. The use of a sodium calcium mixture as a bath brought out some very instructive facts in this connection. Greene<sup>2</sup> and Howell<sup>3</sup> have shown that in ordinary sodium calcium mixtures strips may give series of many hours' duration, and usually characterized by gradually rising tone. In the absence of oxygen the series given in such mixtures were astonishingly similar to ordinary sodium chloride series; there was no marked rise in tone, and the length of the series was about the same—for twelve experiments it averaged fifty-seven minutes, as compared with fifty-one minutes for the sodium chloride series. In the presence of considerable calcium, four times the physiological concentration having been used in one instance, the result did not differ. When oxygen was passed into a sodium calcium solution in which exhaustion had occurred, a very prompt recovery ensued, resembling precisely that which follows the addition of a calcium salt to an ordinary sodium chloride solution in which a strip has become exhausted.

An interesting observation, and one which seems to the author to throw much light on the problem under discussion, is this: strips

<sup>1</sup> MARTIN: This journal, 1904, xi, p. 107.

<sup>2</sup> GREENE: *Ibid.*, 1898, ii, p. 85.

<sup>3</sup> HOWELL: *Loc. cit.*, p. 187.

immersed in a small volume (25 c.c.) of solution, — either pure sodium chloride or a sodium calcium mixture, from which no attempt has been made to remove the oxygen, but enclosed as completely as possible to prevent diffusion from the surrounding air, — exhibit many of the phenomena described above for oxygen-free solutions. In detail, strips under this condition show a somewhat shortened series in sodium chloride solution; they do not recover from sodium chloride exhaustion upon the addition of calcium; they do show recovery when an alkali is added, or when the solution is oxygenated; the series in sodium calcium mixtures are characterized by their short duration, and by the fact that there is no rise in tone.

#### PRESENCE OF OXYGEN.

In the preceding paragraph an account was given of the phenomena attending a lack of oxygen. In the following sections will be described those which accompany a more or less abundant supply of this gas.

**Saturation with pure oxygen.**— When fresh ventricular strips are immersed in  $\frac{7}{8}$  sodium chloride through which a constant stream of pure oxygen is bubbling, they show first a latent period somewhat prolonged as compared with that seen in ordinary sodium chloride, and much prolonged as compared with that which occurs in oxygen-free sodium chloride. The series of contractions in oxygen-saturated saline are invariably many times longer than in the ordinary solution. In the author's experiments the average length of six series in the oxygen-saturated medium was ten hours and ten minutes, while the controls in ordinary sodium chloride showed an average length of slightly less than an hour. One exceptionally long series in oxygen-saturated saline was studied in detail, and the following facts were brought out. The control strip in ordinary  $\frac{7}{8}$  sodium chloride gave 628 contractions in fifty-two minutes, and was then wholly exhausted; the amount of work done by this strip during this time was approximately 1900 gm. mm. The test strip in salt solution kept saturated with oxygen gave 16,755 contractions in thirteen hundred and forty minutes, and was then wholly exhausted; the amount of work done by this strip was approximately 12,000 gm. mm., or more than six times that of the companion strip which did not receive an adequate oxygen supply. The strips were as near alike as they could be cut the control being slightly the heavier.

Strips which have become exhausted in oxygen-saturated saline may be recovered markedly by the addition of a calcium salt to the bath. In the case described above, in which a strip had become exhausted after twenty-two hours activity in an oxygen-saturated bath, enough calcium chloride solution was added to bring the concentration of that substance in the bath up to 0.03 per cent, whereupon the strip promptly recovered in the manner characteristic of treatment with calcium, and continued active for thirteen hours more. It should be stated that the passage of oxygen through the solution was continued after the addition of the calcium salt. Recovery may also be obtained by the addition of an alkali, and this treatment is effective whether or not the oxygen is passed through the solution after the addition of the alkali.

The effect upon strips of saturating with oxygen the solutions in which they have become exhausted was stated in a previous paragraph, but will be considered in detail here for the sake of completeness. After exhaustion in sodium chloride solution saturation with oxygen brings about a gradual increase in vigor, which is precisely similar in appearance to that which follows transfer to a moist chamber. This recovery may be had after exhaustion either in an oxygen-free or in an ordinary solution. After exhaustion in oxygen-free sodium calcium mixture, passage of oxygen into the solution results in a very prompt and marked recovery, usually accompanied by a rise in tone, and referable to the calcium contained in the solution.

A fact having an important bearing upon the problem in hand is that in the long series which occur in oxygen-saturated media, the tissue, after exhaustion of its initial oxygen, seems to become directly dependent upon the external oxygen supply. This is indicated, not only by the long series which follow a large oxygen supply, but by the fact that if the stream of oxygen be cut off the vigor of contraction at once begins to diminish very rapidly, and exhaustion ensues in a few minutes. This exhaustion comes on as quickly in the earlier parts of the series as in the later parts, provided the activity has been long enough continued to exhaust the tissue's initial oxygen, a process which seems to require about an hour. Passing streams of other gases, such as nitrogen or hydrogen, through the solution, is without effect in deferring the onset of exhaustion, showing that the sustaining effect of the stream of oxygen is not due to its action in sweeping away impurities.

**Saturation with atmospheric oxygen.**—When a stream of air is



passed through a liquid the amount of oxygen which goes into solution is doubtless somewhat greater than the liquid takes up as the result of simple exposure to the atmosphere, but only one-fifth as great as the amount taken up when pure oxygen is passed through it, because of the difference in pressure between oxygen gas and the oxygen of the air. In view of this difference in oxygen content under the two conditions, it was with great interest that the observation was made that every one of the phenomena described above for oxygen-saturated solutions could be duplicated with air-saturated solutions. That is to say, that as regards the long latent period, the extremely long sodium chloride series, and recovery from ordinary sodium chloride exhaustion, air saturation is quite as effective as saturation with pure oxygen. The meaning of this observation will be discussed in its proper place. It is sufficient to state here that in the opinion of the author the saturation point of the tissue for oxygen is reached at about the saturation point of the solution for oxygen at its partial pressure as exerted in the air, and much below its saturation point for pure oxygen gas.

Two observations were made with air-saturated solutions which were not made with oxygen-saturated media, but the nature of these observations, as well as the close similarity shown by the phenomena in which comparisons were made, renders it safe to conclude that the effects shown were due to oxygen. The first of these observations had to do with the effect of a preliminary calcium bath upon the latent period. In the absence of oxygen such a bath seems to be without effect; in an ordinary solution the tendency is to shorten the latent period markedly; in an air-saturated solution the preliminary calcium bath also shortened the latent period, and by as large a fraction as in the case of the ordinary solution. The second observation was of the effect of air saturation on sodium calcium mixtures. In the absence of oxygen the series in such a mixture is short and without rising tone. When such a mixture is saturated with air the series is also short, but exhibits markedly rising tone which passes finally into what Howell<sup>1</sup> has called calcium rigor.

**Use of a large volume of solution with free access of air.**— Usually experiments with heart strips are carried on under the conditions named in the heading of this paragraph, namely, a relatively large volume of the bathing medium whose surface is open freely to the air. These conditions are intermediate between those in which

<sup>1</sup> HOWELL: *Loc. cit.*, p 187.

oxygen is lacking and those in which it is present in abundance. As might be expected, strips immersed in such solutions exhibit phenomena somewhat intermediate between those seen under the other conditions. The latent period in  $\frac{2}{3}$  sodium chloride tends to be long, a preliminary calcium bath shortens it; the sodium chloride series is longer than in oxygen-free solutions, but very much shorter than in oxygen- or air-saturated solutions. Good recovery from sodium chloride exhaustion may be had by the addition of an alkali, or a small proportion of a calcium salt, or by furnishing an adequate supply of oxygen. This latter may be done by direct oxygenation of the solution or by transferring the strip to moist air. The best possible recovery results from a combination of the calcium treatment with thorough oxygenation. Strips in sodium calcium mixtures also show series under these conditions intermediate between those in oxygen-free and oxygen-saturated media. They are usually much longer than in either of the latter solutions, probably because the calcium in the solution has opportunity to become effective, which it seems unable to do in oxygen-free solution, and its effect does not become excessive as it tends to in oxygen-saturated solution.



Diagrammatic figure to illustrate the influence of oxygen on the duration of the sodium chloride series. *A* represents the length of the series in oxygen-free saline. *B* represents the length of the series in ordinary saline. *C* represents the length of the series in oxygen-saturated saline. Each space stands for one hour.

#### THE RELATION OF CALCIUM TO THE OXYGEN SUPPLY.

Examination of all the experiments reported above in which a calcium salt was used, shows that the stimulating effect of calcium manifested itself only when the tissue was in an environment containing more or less oxygen. A preliminary calcium bath, which shortens the latent period in the presence of oxygen, did not do so in an oxygen-free bath. This, perhaps, is no more than would be expected in view of the very short latent period which occurs anyway in oxygen-free saline, and taken by itself may have little significance; it falls in line, however, with the remaining observations, and so is included. The tendency of calcium to improve the beat of strips after exhaustion in sodium chloride was also dependent upon the presence of external oxygen. In addition to the experiments show-

ing this, which have already been described, some special experiments were performed to test this point. In a former paper<sup>1</sup> the author described the remarkable improvement in beat which follows moistening with a calcium-containing solution a strip which is beating feebly in moist air. To determine whether this calcium effect is dependent on the presence of external oxygen, strips were immersed in sodium chloride solution till exhausted; the liquid was then drawn off and replaced with nitrogen gas, a constant stream of which was kept bubbling through the enclosing tube. In such nitrogen moist chambers strips might continue to beat very feebly for a long time, but moistening with a calcium-containing liquid never caused any improvement in beat, except occasionally a slight one for a few contractions. Companion strips, however, after precisely the same treatment, except that they were in moist air, showed marked and lasting improvement in beat following the application of a calcium-containing solution. The stimulating influence of calcium, after the extremely long series in oxygen-saturated saline, was so marked as to deserve special comment. In this case a strip had been active for more than twenty-two hours, and its energy output had been fully six times that of strips in ordinary sodium chloride exhaustion. The improvement in beat which resulted from the calcium treatment was, however, quite as marked and well sustained as is usual after ordinary exhaustion.

Most striking of all the phenomena in this connection, however, was the remarkable ineffectiveness of the calcium in an oxygen-free sodium calcium mixture. The appearance of the initial series in such a mixture was so closely similar to that of an oxygen-free sodium chloride series, that one is forced to the conclusion that the tissue was not responding to any influence of the calcium. When oxygen was passed into the solution, however, the characteristic calcium influence became at once apparent.

Quite a different view of the relation between calcium and the oxygen supply, but one, perhaps, equally instructive, is afforded by the conduct of fresh strips in sodium calcium mixtures through which a stream of air is bubbling. Here we have conditions in which the calcium may act most freely, and, as might be expected, its influence under these circumstances tends to become excessive. A concentration of calcium, which, in an ordinary solution, would be very beneficial, becomes, in an air-saturated solution, quite detrimental.

<sup>1</sup> MARTIN: *Loc. cit.*, p. 126.

The usual method of judging whether the calcium effect is excessive is by observing how marked is the tendency of the strip to go into calcium rigor. As we have seen, the concentration of calcium which brings on this condition in a given time in an air-saturated solution is much smaller than that required to produce it in the same time in an ordinary solution.

Before leaving this topic some mention should be made of the influence of the oxygen supply on the tone changes caused by calcium. In general it may be said that although the tendency of calcium to cause increased tone is largely dependent upon the presence of an external oxygen supply, it is not wholly so. The addition of calcium to an oxygen-free sodium chloride solution in which exhaustion has taken place does not result in improvement of the beat, but usually causes a considerable rise in tone. On the other hand, as has been previously stated, in oxygen-free sodium calcium mixtures there is usually practically no increase in tone.

#### THE RELATION OF ALKALI TO THE OXYGEN SUPPLY.

Merunovicz<sup>1</sup> appears to have been the first observer to note the favorable influence exerted on the heart by alkali. Gaule<sup>2</sup> and others confirmed the observation of Merunovicz, and interpreted it as meaning that the alkali neutralizes the toxic carbon dioxide which is formed during the activity of the tissue, and which is hindering it from doing its best work. More recently Benedict<sup>3</sup> has laid great stress upon this alkali effect. He interprets it, however, upon the simple assumption that the alkali acts by causing an increase in the tone of the tissue, taking the ground that sodium chloride exhaustion is due to excessive loss of tone, and that whatever causes a rise in tone is likely to bring about a recovery.

The author has found considerable difficulty in drawing conclusions from his experiments with alkali, chiefly because there appears to be for each heart a certain concentration of alkali at which it gives its most typical response, whereas under other doses it may give quite abnormal responses. Thus if the concentration of alkali is less than the optimum the tissue may show no signs of its influence, and, on the other hand, if the concentration is much greater, a distinctly

<sup>1</sup> MERUNOVICZ: *Arbeiten an der physiologischen Anstalt zu Leipzig*, 1875, p. 132.

<sup>2</sup> GAULE: *Archiv für Physiologie*, 1878, p. 291.

<sup>3</sup> BENEDICT: *This journal*, 1905, p. 196.

injurious effect may appear. When the additional statement is made that the most favorable alkali concentration is apt to be different for different hearts, it will be understood that it has not always been easy to decide whether a certain observation represented the most typical response of the tissue to the condition under examination or not. However, certain facts with regard to the relation of alkali to the activity of the tissue seem sufficiently well established to be put on record. In the first place, as has been already stated, the ability of alkali to cause recovery after sodium chloride exhaustion is not dependent upon the presence of external oxygen; a perfectly typical alkali recovery follows the addition of a small proportion of sodium carbonate to an oxygen-free sodium chloride solution in which a strip has become exhausted. Another fact, which seems to the author to be very significant, is that the series which result from the use of alkali present certain features which are perfectly characteristic, which depend on the action of the alkali, and whose absence, in a strip surrounded by an alkaline medium, indicates that for some reason the alkali influence is not effective. These characteristic features are two: 1st, a series of considerable and almost constant length; in all the author's experiments the length of series approximated twenty hours: 2d, a comparatively feeble beat; the greatest extent of contraction under alkali influence was never as much as one-half that shown by the same strips at the beginning of activity in sodium chloride solution. In the author's experiments alkali series showing these characteristics were obtained under the following different conditions: immersing a fresh strip in oxygen-free alkaline sodium chloride resulted in a typical alkali series; this was noteworthy because of the fact that the beat was feeble from the outset, whereas series in neutral oxygen-free saline begin with as great vigor as in ordinary saline; addition of sodium carbonate to an oxygen-free salt solution in which exhaustion had occurred resulted in a typical alkali series; addition of alkali to an ordinary salt solution under the same conditions produced the same effect; so also did the addition of an alkali to an oxygen-saturated sodium chloride solution in which a strip had given the usual long series. It will be noted that in each of these instances the alkali effect manifested itself upon a strip which was either in a condition of so-called "exhaustion," or was deprived of an external supply of oxygen. The following observations will show that it is apparently only under such conditions that the characteristic series described above accompany the presence of alkali in the bath.



A fresh strip was immersed in ordinary alkaline sodium chloride solution. The series of contractions given by it presented at first the characteristics of series in neutral sodium chloride, namely, considerable initial vigor, and a continuous decline in extent of contraction. Instead of proceeding to complete exhaustion, however, the decline in vigor ceased when the extent of contraction had become something less than one-fourth that at the beginning, and the series proceeded in the manner characteristic of strips under alkali influence, coming to an end in about twenty-three hours after the beginning of activity, and twenty-two hours after the specific alkali influence began to be apparent. Another, and even more instructive, experiment was as follows: a fresh strip was immersed in an alkaline salt solution through which a constant stream of air was bubbling; contractions began in a half hour, and continued for nearly nine hours in the manner characteristic of strips in air-saturated saline, differing only from such series in that there appeared to be a rather more pronounced tendency for the strip to go into a state of tone than is usual under such treatment; at the end of this time, and while the strip was still beating with considerable vigor, the air was shut off; previous experience had shown that in neutral solutions of this sort, shutting off the air after several hours activity would result in a rapid decline of vigor and complete arrest within ten or fifteen minutes; in the alkaline solution, however, the result of cutting off the flow of air was a prompt and marked fall in tone, and an immediate change of the character of the series to that which has been already described as indicating that the alkali influence is effective; this series continued for about fifteen hours; at the end of that time, although the strip was not entirely exhausted, the air was once more turned into the solution; this treatment resulted in a good recovery, with rising tone, and the strip continued active under the influence of the abundant air supply for ten hours more. These experiments seem to show that, whereas a heart strip may under proper conditions show an improvement in beat as the result of the action of an alkali, this effect is not only not dependent, as is the calcium effect, on the presence of external oxygen about the tissue, but is rather held in abeyance so long as the needs of the tissue for oxygen are met from that source, making its influence felt when the external oxygen supply becomes deficient.



## CONCLUSIONS.

In the opinion of the author the experiments described in the preceding paragraphs of this paper throw much light on some of the phenomena exhibited by heart muscle, regarding whose meaning there have been diverse views.

**The sodium chloride arrest.**—Let us consider first the exhaustion which comes on after a series of contractions in sodium chloride solution. Various assumptions have been advanced to explain it. Loeb<sup>1</sup> attributed it to the poisonous action of sodium ions. Howell<sup>2</sup> suggested that it might be due to lack of calcium, as the result of the loss of that substance by diffusion; the same view was taken by the author in a previous paper.<sup>3</sup> Lingle<sup>4</sup> held that lack of oxygen might be responsible for the exhaustion, although in the same paper he advanced arguments in support of Loeb's assumption. Finally Benedict<sup>5</sup> has advanced the idea that excessive loss of tone is the cause of the arrest. The experimental data herein recorded seem to necessitate modification of whichever of these views may be held. Sodium chloride arrest was obtained under three conditions, namely, deficiency of oxygen, a moderate supply of oxygen, and abundance of oxygen. It seems highly probable that the exhaustion in oxygen-free saline may be directly due to lack of oxygen. The ease with which activity is renewed when the solution is ærated, and the fact that treatment with calcium is ineffective, are arguments in favor of this view. The fact that addition of an alkali will cause recovery in the absence of oxygen may seem incompatible with it, but to the author's mind it is not necessarily so, as will be shown presently. In view of this probability, and in order that a convenient starting-point may be had, from which to proceed to the interpretation of the other cases of sodium chloride arrest, the author makes the assumption that the series of beats given by a heart strip in oxygen-free saline is wholly at the expense of the oxygen in solution in its juices, and that arrest marks the point at which this store of oxygen is consumed.

When we pass to the case of the arrest in an ordinary saline solution, that is, in the presence of a moderate oxygen supply, phenomena

<sup>1</sup> LOEB: This journal, 1899, iii, p. 327.

<sup>2</sup> HOWELL: *Loc. cit.*, p. 184.

<sup>3</sup> MARTIN: *Loc. cit.*, p. 134.

<sup>4</sup> LINGLE; *Loc. cit.*

<sup>5</sup> BENEDICT: *Loc. cit.*

are encountered which differ only in one important respect from those accompanying an oxygen deficiency. This difference is in the response toward calcium. Inasmuch as an excellent recovery can be obtained after exhaustion in ordinary sodium chloride solution by the mere addition of a little calcium chloride, it can scarcely be supposed that the exhaustion is due solely to lack of external oxygen. Equally untenable is the view that the arrest is altogether due to loss of calcium by diffusion, since thorough æration of the solution suffices to bring about recovery. A satisfactory explanation of the arrest can be had, however, if it be assumed that it is due, not to the lack of external oxygen, but to the inability of the tissue to absorb it at the low concentration at which it is present in an ordinary solution. The recovery which follows æration would indicate that as the result of thorough saturation with air the oxygen content of the solution was raised to a point at which the tissue is again able to take it up; the recovery which results from the addition of a calcium salt would be taken to mean that the power of the tissue to absorb oxygen is to a certain extent dependent upon the presence of diffusible calcium, so that when such calcium is furnished in sufficient amount, the tissue can supply itself with oxygen from the small concentration of the gas present in the solution. Transfer to moist air, which is especially efficacious in bringing about recovery, not only surrounds the tissue with abundance of oxygen, but at the same time cuts off all further loss of calcium, and so seems to bring the strip into a very favorable condition for prolonged activity. According to this view, then, the arrest in ordinary sodium chloride solution is due to a species of asphyxia resulting from the loss by diffusion of the calcium ions upon which the power of the tissue to take up oxygen from the surrounding medium depends.

The third case of arrest, that in oxygen-saturated sodium chloride, falls in readily with the assumption just made. Here the oxygen concentration is maintained at a higher level than in the previous case, and it would be expected that a much greater loss of calcium ions must occur before the tissue loses the power to absorb it; the long series of beats would be looked upon as marking the time required for the calcium diffusion to reach the point beyond which no farther oxygen absorption is possible. The excellent recovery which follows the addition of a calcium salt is just what should occur if this view be correct, inasmuch as there is abundance of oxygen about the tissue, whose ready absorption is promoted by the addition of the calcium salt to the bath.

Neither the assumption of Loeb that the arrest is due to a poisonous influence of the sodium ions, nor that of Benedict that it results from excessive loss of tone, can be readily reconciled with the great increase in length of series which results from supplying abundant oxygen. To be sure, those who take Loeb's position might maintain that oxygen counteracts somewhat the poisonous effect of the sodium, and so prolongs the series, but, as has been frequently pointed out, the average mind finds it easier to adopt a view which assigns positive rôles to the different substances which affect the heart, rather than one which makes sodium the only positive agent, and assigns to all others negative rôles. Benedict, also, might hold that saturation with oxygen tends to keep the tone of the tissue from falling off too much, and so is effective in prolonging the series; but to the author's mind it is much easier to look upon the tone changes which accompany the responses of heart strips to various treatments as in the nature of effects rather than causes.

**The rôle of calcium.**— In the experimental part of this paper the author called attention repeatedly to the remarkable ineffectiveness of calcium in the absence of oxygen. This was particularly noteworthy in the case of fresh strips immersed in oxygen-free sodium calcium mixtures, and of strips placed in a nitrogen atmosphere after exhaustion in sodium chloride and moistened with calcium-containing solutions, but was also conspicuous in every case of the use of calcium. There is, then, undoubtedly a close interrelation between the stimulating influence of calcium and the oxygen supply of the tissue. This might be explained by the simple assumption that calcium acts as a direct stimulant upon the tissue, but that the production of vigorous contractions by the latter is absolutely dependent upon the presence of sufficient oxygen, so that no matter how strong a stimulus is applied, in the absence of oxygen no vigorous response can be given. The author has been led to reject this simple view in favor of the one proposed by him in the preceding paragraphs, namely, that an important rôle of the calcium is to promote the absorption of oxygen by the tissue, chiefly from a consideration of the influence of oxygen upon sodium chloride arrest. If we take the view that calcium acts wholly as a direct stimulant, we must necessarily attribute the arrest in ordinary sodium chloride to deficiency of oxygen rather than to lack of calcium, because of the fact that when oxygen is supplied a long-continued recovery ensues, implying that enough calcium must still be present in the tissue to furnish the required stimulus. If this is so, however,

it is difficult to explain why the addition of calcium should bring about such an excellent recovery; a strip which contains enough calcium to induce a long series of beats, but is held in arrest through deficiency of oxygen, would scarcely be expected to benefit much from an increase in its calcium supply. This difficulty disappears if it be assumed that the most urgent demand of the tissue is for oxygen, and that the calcium ions perform the minor function of promoting its absorption. According to this assumption, the calcium requirement of the tissue would vary in an inverse relation to its oxygen supply, and the result of supplying calcium to a strip in ordinary sodium chloride exhaustion would be to so far improve its ability to absorb oxygen as to compensate for the meagreness of the supply. This conception should not be carried so far as to imply that a point of oxygen saturation might be reached at which the presence of calcium could be altogether dispensed with. The author's thought inclines rather to the view that the absorption of oxygen by heart tissue is impossible except in the presence of a certain amount of calcium; a view which is supported by the phenomena exhibited by strips in oxygen-saturated saline. Arrest in this medium cannot be due to deficiency of oxygen, inasmuch as no deficiency exists, and so must be due to lack of calcium. Conditions are here peculiarly favorable for a very large reduction of the calcium content of the tissue, because of the long-continued opportunity for diffusion. The very excellent recovery which follows a renewal of the calcium supply under these conditions seems to indicate strongly that its beneficial action is intimately associated with the absorption of oxygen by the tissue.

According to the assumption under discussion, the phenomenon known as "calcium rigor," a condition of extreme tone into which strips in oxygenated sodium calcium mixtures tend to go, is to be ascribed rather to over absorption of oxygen, under the influence of the calcium, than to a direct action of the calcium itself. The fact that a very considerable percentage of calcium in the solution does not show any marked tendency to induce rigor when oxygen is excluded, is a point in support of this idea. It must be granted, however, as was shown in these experiments, that calcium does exert a certain direct influence upon the tissue, tending to cause rise in tone, and that under proper conditions, especially when the calcium concentration is high, this effect will be produced whether oxygen is present or not.

**The rôle of alkali.** — The phenomena which result from the application of an alkali to heart strips, and in which the alkali influence is manifest, are so simple and uniform in character as to lead the author to hope that a simple explanation of the rôle of alkali may prove to be the true one. The most conspicuous characteristics of the alkali effect are: 1st, that it seems to be wholly independent of the presence of external oxygen; 2d, that the entire output of energy of a strip beating under the influence of alkali seems to tend to be proportional to the mass of the strip. This second characteristic is, of course, only a rough approximation based upon the facts that the lengths of alkali series were nearly the same in all the author's experiments, and that the height of contraction seemed to be somewhat proportional to the size of the strip. The unknown factors determining the condition of nutrition of different hearts are so many that only the loosest approximate estimation of the possible energy output of any given piece of tissue can be made; but having this all in mind, the uniformity of the alkali series was so striking as to encourage the author to make the suggestion that the way in which alkali brings about its characteristic series of contractions is by inducing the decomposition of some oxygen-containing compound in the tissue, which is not affected by other treatments, and from which is furnished the oxygen necessary for the resultant activity. This supposition explains, better than any other which has occurred to the writer, how alkali can be effective in the absence of external oxygen; it also shows how an alkali series can be "slipped," so to speak, into the middle of a series in air-saturated saline. According to this supposition a strip which is brought under the influence of an alkali might yield its entire store of available intramolecular oxygen without impairing thereby its ability to absorb external oxygen through the influence of calcium; the two processes being largely, if not wholly, distinct. The experimental basis for this supposition is rather suggestive than demonstrative at present, but it seemed to the author worth advancing, in the hope that it might stimulate further examination into the subject.

The discussion of the phenomena herein recorded has confined itself to their possible interpretation as seen in isolated ventricular strips. The author hopes in a future paper to show that they throw light also on the normal activity of the intact living heart. Certain of the phenomena described in this paper, notably those which have



to do with the latent period, have been omitted from this discussion with the expectation of taking them up at that same future time.

#### SUMMARY.

1. Fresh ventricular strips, immersed in oxygen-free sodium chloride solution, give series of contractions which are characterized by their comparatively short duration; the assumption is made that under these conditions the tissue draws wholly upon the oxygen in solution in its juices to obtain the amount necessary for its activity. The arrest which ends the series is supposed to be due to asphyxia resulting from exhaustion of the oxygen supply. It is characterized by the ease with which oxygenation induces recovery, and by the fact that addition of a calcium salt is without effect.

2. Exhaustion in a saline bath having in solution a moderate amount of oxygen is assumed to be due, not to lack of oxygen, but to the fact that the ability of the tissue to absorb it has diminished to such a degree that it can no longer do so at the low concentration at which the oxygen is present in the solution.

3. The very long series of beats which occur in sodium chloride solution kept saturated with oxygen, show that the tissue possesses the power to take up oxygen from the surrounding medium, provided it is present therein in sufficient concentration. The continual decline in vigor, and the arrest which ultimately ensues, show that as the result of the prolonged immersion something is lost by the tissue upon which its power to absorb oxygen depends.

4. The difference between the oxygen concentration at which the phenomena of oxygen deficiency occur, and that at which those of oxygen abundance are seen, is comparatively small.

5. The excellent recovery which follows the addition of a small proportion of a calcium salt to an oxygen-containing solution in which exhaustion has occurred, indicates that the ability of the tissue to absorb oxygen from the surrounding medium is dependent upon the presence within it of calcium ions. It is assumed that the chief function of calcium is to promote oxygen absorption, a view which is supported by the close interrelation which is shown to exist between the stimulating influence of calcium and the oxygen supply.

6. Whenever a strip is placed in an alkaline medium under such conditions that it becomes responsive to the alkali influence, a very typical series of contractions is given, in which the total energy



output of the tissue seems to bear a definite relation to its mass. This energy liberation is entirely independent of the supply of external oxygen. It is suggested that the effect of the alkali is to decompose some oxygen-containing compound in the tissue, thereby rendering available a certain definite store of oxygen which is not affected by other modes of treatment.

## THE MECHANISM OF SALT GLYCOSURIA.

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**E**XPERIMENTAL glycosuria may be indicative of one of several phases of abnormal carbohydrate metabolism. It may indicate a condition of nervous derangement as in the *piqûre* diabetes of Claude Bernard, or according to Minkowski its appearance may denote injury to the pancreas. Phlorhidzin and uranium glycosuria are symptoms of disturbances of renal integrity, while the appearance of sugar in the urine after administration of various anæsthetics and drugs<sup>2</sup> is but a manifestation of deranged carbohydrate metabolism induced by interference with respiratory processes.

Coincident with the appearance of the several types of glycosuria mentioned quantitative changes in the sugar content of the blood may be demonstrated. Those cases of glycosuria where the sugar content of the blood has been ascertained have shown that with the exception of the diabetes induced by phlorhidzin and uranium salts, the quantity of sugar in the blood is above normal — that hyperglycæmia is in evidence. When phlorhidzin and uranium salts cause glycosuria, hypoglycæmia is observed. This form of glycosuria has been looked upon as being of renal origin, the kidney being injured by the drugs named in such a manner that it is no longer capable of preventing the egress of sugar from the blood. When the kidneys have been injured in the manner indicated, the sugar appearing in the urine has merely leaked out from the blood, as it were, with consequent hypoglycæmia. On the other hand, when hyperglycæmia has been noted, it is probable that this condition is brought about by one of two circumstances, namely, either an increased formation of sugar or a decreased consumption. Either condition promotes an excess of sugar in the blood which the body endeavors to decrease to the normal. Consequently the excess of sugar appears in the urine.

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<sup>2</sup> UNDERHILL: *Journal of biological chemistry*, 1905, i, p. 113, where references to the earlier literature are given.

## SALT GLYCOSURIA.

In 1871 Bock and Hoffmann<sup>1</sup> found that large amounts of a one per cent solution of sodium chloride injected into the arterial circulation of rabbits produced diuresis, which was followed by the appearance of sugar in the urine. Külz<sup>2</sup> succeeded in inducing similar effects, not only with sodium chloride, but also with solutions of sodium acetate, sodium carbonate, sodium valerianate, and sodium succinate.

Recently these experiments have been repeated and extended by Martin Fischer. In his preliminary observations Fischer<sup>3</sup> modified somewhat the method of injection followed by Bock and Hoffman in that he introduced the solutions into the lateral vein of the ear of the rabbit instead of directly into the arterial circulation. The results obtained corroborate those of the earlier investigators, and further show that when glycosuria has been set up it may be inhibited or arrested by subsequent injection of a mixture of the sodium salt and calcium chloride. The replacement of the calcium solution by that of a sodium salt after the glycosuria has ceased causes a reappearance of sugar in the urine. It has also been shown by Fischer that the rapidity with which sugar appears in the urine varies directly with the concentration of the salt solution employed, that the degree of diuresis bears no definite relation to the quantity of sugar excreted, and that albuminuria is a frequent accompaniment of this glycosuria.

Brown<sup>4</sup> obtained results very similar to those of Fischer, although the latter discounts their value, since Brown used an anæsthetic throughout his experiments which of itself is capable of calling forth glycosuria.

The later papers of Fischer<sup>5</sup> are concerned mainly with an attempt to account for the mechanism involved in the production of salt glycosuria. He observes that when sodium salts are injected directly into the arterial system (into the axillary artery, hence through the vertebral arteries to the spinal bulb), glycosuria may be demonstrated much sooner than when the injection has been made

<sup>1</sup> BOCK and HOFFMANN: *Archiv für Anatomie und Physiologie*, 1871, p. 550.

<sup>2</sup> KÜLZ: *ECKHARD'S Beiträge*, 1872, vi, p. 117 (cited from E. PFLÜGER: *Archiv für die gesammte Physiologie*, 1903, xcvi, p. 313).

<sup>3</sup> FISCHER: University of California publications, physiology, 1903, i, p. 77, and *ibid.*, 1904, i, p. 87.

<sup>4</sup> BROWN: *This journal*, 1904, x, p. 378.

<sup>5</sup> FISCHER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 80, and *ibid.*, 1905, cix, p. i.

into the venous circulation. From these facts the conclusion is drawn that in the glycosuria provoked by the introduction of sodium salts into the circulation two factors are involved,—one an action upon the kidney by which diuresis is induced, the second an influence upon the diabetic centre in the spinal bulb which causes glycosuria.

To the writer the deductions made by Fischer from the data presented seem hardly conclusive. The fact that glycosuria may appear sooner when salt solution is injected into the arterial system than when it is introduced into a vein, is insufficient evidence that the salt exerts a specific action upon the spinal bulb in stimulating a diabetic centre; for other possibilities are readily suggested. Neither is there any evidence in Fischer's work that the glycosuria induced by injection into a vein is of the same type as that caused by introduction into an artery, although he assumes that they are identical. It seemed to the writer that a much more satisfactory and convincing proof of the mechanism of salt glycosuria was to be found in studying the quantitative changes of the sugar content of the blood. This has been done, and the results obtained have been made the subject of the present paper.

#### THE MECHANISM OF THE GLYCOSURIA FOLLOWING INJECTIONS OF SODIUM CHLORIDE INTO THE VENOUS CIRCULATION.

Fischer has shown that when sodium chloride solutions are rapidly introduced into the ear vein of the rabbit the rate of onset of glycosuria is proportional to the concentration of the salt solution. For example, when a solution of  $\frac{m}{6}$  sodium chloride is injected into the venous circulation at the rate of 75 to 100 c.c. of solution per fifteen minutes, polyuria is in evidence in from ten to fifteen minutes after the beginning of the injection, and sugar appears in the urine about two hours after the beginning of the injection. When a more concentrated sodium chloride solution, for example,  $\frac{m}{2}$  sodium chloride, is employed, sugar appears in the urine after 20 to 30 c.c. have been introduced.<sup>1</sup> Solutions less concentrated than  $\frac{m}{6}$  sodium chloride fail to produce glycosuria, although polyuria may be observed. When glycosuria has been established by saline infusion, the excretion of sugar may be markedly lessened or entirely inhibited by substituting for the salt solution a mixture of sodium and calcium chloride.

The facts that sodium chloride solutions injected intravenously

<sup>1</sup> Personally communicated (F. P. U.).

induce polyuria and glycosuria; that the glycosuria appears more rapidly the greater the concentration of the salt solution; that the excretion of sugar may be lessened or inhibited by substitution of a mixture of sodium chloride and calcium chloride, and the introduction of the latter is followed by a temporary fall in the amount of urine excreted per unit of time, — point to an increased permeability of the kidney as the factor involved. J. B. MacCallum<sup>1</sup> has shown that salt diuresis in rabbits may be inhibited or lessened by calcium chloride, and Sollmann<sup>2</sup> has demonstrated in his perfusion experiments that calcium chloride will decrease the permeability of the kidney for sodium chloride both in the living and dead organ. Moreover, the permeability of the excised kidney varies directly with the concentration of the salt solution; that is, changing from weaker to stronger solutions of sodium chloride causes an increased vein and ureter flow, and vice versa.<sup>3</sup> In view of these observations the writer believes that the action of the salt upon the kidney affords a more probable explanation of the glycosuria induced by injections into the ear vein of the rabbit than does the assumption of a specific influence upon the nervous system. If the glycosuria is due to an increased permeability of the kidney, it is reasonable to assume that the sugar content of the blood will be below normal. The dilution of the blood by large volumes of injected fluid is a factor that must also be taken into account in studying the sugar content, for although the quantity of fluid excreted is approximately equal to the volume injected, the introduction of hyperisotonic solutions tends to dilute the blood.

The foregoing considerations have therefore been subjected to experimental investigation. In introducing the salt solution into the venous circulation the excellent method employed by Fischer was followed. The content of sugar in the blood was determined according to the method recommended by Vosburgh and Richards,<sup>4</sup> and satisfactorily employed in former studies on the blood sugar.<sup>5</sup> Urine was obtained either by catheterization or by expression of the bladder. To determine approximately the dilution of the blood, hæmoglobin

<sup>1</sup> J. B. MACCALLUM: University of California publications, physiology, 1904, i, p. 81.

<sup>2</sup> SOLLMANN: A personal communication from Professor SOLLMANN (F. P. U.). See also this journal, 1905, xiii, p. xv.

<sup>3</sup> SOLLMANN: This journal, 1905, xiii, p. 278.

<sup>4</sup> VOSBURGH and RICHARDS: This journal, 1903, ix, p. 35.

<sup>5</sup> UNDERHILL: *Loc. cit.*

estimations were made before and at the end of the experiment. A typical experiment follows.

TABLE I.

Experiment 5. — Injection fluid:  $\frac{1}{2}$  molecular sodium chloride.

Time.	Volume of salt solution injected in last interval of time.	Volume of urine excreted in last interval of time.	Remarks.
a. m.	c.c.	c.c.	
....	..	..	Rabbit of 2500 gm. No sugar in urine.
11.00	..	..	Hæmoglobin in blood = 55-60 per cent.
11.08	..	..	<i>Injection into ear vein begun.</i>
11.14	50	15	No sugar in urine.
11.17	20	15	Trace of sugar in urine.
11.20	20	22	Large amount of sugar. <i>Injection stopped.</i>
11.23	..	26	Large amount of sugar.
11.26	..	16	" " "
11.27	..	..	" " "
....	..	..	Hæmoglobin in blood = 50-55.
11.32	..	18	Large amount of sugar.
....	..	..	Blood drawn for sugar analysis.
....	..	..	<b>Sugar content of blood = 0.05 per cent.</b>

Five similar experiments yielded entirely concordant results. Estimation of the normal quantity of sugar in rabbit's blood gave 0.167 per cent. Rose<sup>1</sup> found that the sugar content of rabbit's blood varies from 0.15 to 0.20 per cent. The results given above indicate that dilution of the blood is insufficient to account for the subnormal quantity of sugar, for while the dilution is at most one-tenth, the diminution of the sugar is at least two-thirds.

If the increased permeability of the kidney is responsible for the appearance of glycosuria at the expense of the sugar content of the blood, it should follow that after the decrease in sugar, excretion by injection of a mixture of sodium chloride and calcium chloride, the

<sup>1</sup> ROSE: Archiv für experimentelle Pathologie und Pharmakologie, 1903, xxx, p. 15.



sugar content of the blood should regain the normal. The following typical experiment bears upon this point. The details of similar experiments need not be given here.

TABLE II.

*Experiment 19.*—Injection fluid:  $\frac{1}{8}$  molecular sodium chloride followed by a solution containing 975 c.c.  $\frac{1}{8}$  molecular sodium chloride + 25 c.c.  $\frac{2}{3}$  molecular calcium chloride.

Time.	Volume of salt solution injected in last interval of time.	Volume of urine excreted in last interval of time.	Remarks.
a. m.	c. c.	c. c.	
....	..	..	Rabbit of 2280 gm. No sugar in urine.
10.30	..	..	Hæmoglobin in blood = 50-55 per cent.
10.35	..	..	<i>Injection of sodium chloride into ear.</i>
10.40	40	0	
10.45	23	0	
10.50	13	16	No sugar in urine.
11.00	44	16	" " "
11.05	33	11	" " "
11.10	35	14	" " "
11.15	37	17	" " "
11.20	29	22	" " "
11.25	28	20	" " "
11.30	37	24	" " "
11.35	31	26	" " "
11.40	30	30	" " "
11.45	50	34	" " "
11.50	50	42	Trace of sugar in the urine.
11.55	42	30	<i>Injection stopped.</i> Sugar in urine.
12.00	..	30	Sugar in urine.
p. m.	..	..	
12.05	..	..	<i>Injection of calcium mixture into ear vein.</i>
12.10	53	23	Large quantity of sugar in urine.
12.15	30	26	" " " "
12.20	40	30	" " " "

TABLE II. (continued).

Time.	Volume of salt solution injected in last interval of time.	Volume of urine excreted in last interval of time.	Remarks.
p. m. 12.25	c.c. 34	c.c. 38	Large quantity of sugar in urine.
12.30	26	32	" " " "
12.35	29	34	" " " "
12.40	33	31	" " " "
12.45	27	24	" " " "
12.50	27	34	" " " "
12.55	32	34	" " " "
1.00	30	33	" " " "
1.05	39	33	" " " "
1.10	35	33	" " " "
1.15	29	40	" " " "
1.20	32	36	" " " "
1.25	32	32	" " " "
1.30	40	33	" " " "
1.35	40	33	" " " "
1.40	23	34	" " " "
1.45	23	34	" " " "
1.50	42	28	" " " "
1.55	43	40	" " " "
2.00	36	30	" " " "
2.05	36	30	Trace of sugar in the urine.
2.10	36	30	" " " "
2.15	45	30	Mere trace of sugar in urine.
2.20	..	..	Injection stopped.
2.25	..	..	Blood drawn for sugar analysis.
..	..	..	Hæmoglobin = 35-40 per cent.
..	..	..	Sugar content of blood = 0.167 per cent.

From the results outlined it is probable that the mechanism of the glycosuria induced by injections of sodium chloride into the venous circulation of rabbits may be referred to an increased permeability of the kidney, and not to any specific action upon the central nervous system.

#### THE MECHANISM OF THE GLYCOSURIA FOLLOWING INJECTIONS OF SODIUM CHLORIDE INTO THE CEREBRAL ARTERIAL CIRCULATION.

It has been shown by Fischer that when sodium chloride solutions are injected into the cerebral arterial circulation, glycosuria follows much more rapidly than when similar solutions have been introduced into the ear vein. From this observation Fischer was led to formulate the view that the more rapid appearance of sugar in the urine is due to the direct action of the salt solution upon the diabetic centre in the medulla. The solution was injected into an artery leading directly to the medulla, thus giving the salt opportunity to act upon the bulb before entering the general circulation.

In our comparable experiments the salt solution was injected directly into the carotid artery. The artery was rapidly exposed under slight ether anæsthesia, the animal being allowed to recover from the effects of the anæsthetic before the experiment was begun. Control trials indicated that no glycosuria or change in the sugar content of the blood was induced by the brief period of anæsthesia and the operative procedure employed.<sup>1</sup> An illustrative table is shown in Experiment 10.

The experimental data show that the sugar content of the blood is greatly increased. These results are divergent from what is noted after injections of salt solutions into the ear vein. In the case of injections into the ear vein hypoglycæmia is observed, while injections into the carotid artery produce hyperglycæmia. Obviously the mechanisms calling forth these divergent effects cannot be identical. Further evidence that two factors must be considered is derived from Fischer's failure to succeed in inhibiting or lessening, by a mixture of sodium chloride and calcium chloride, the glycosuria which previous injections of sodium chloride into the cerebral arterial system produced. Boçk and Hoffmann and Fischer found that when the injections are made into the arterial circulation polyuria is manifested. The former employed salt solutions of a strength of one per cent,

<sup>1</sup> UNDERHILL: *Loc. cit.* Dogs under slight ether anæsthesia.

while Fischer used  $\frac{m}{2}$  to  $\frac{m}{1}$  sodium chloride solutions. Under similar conditions we have never obtained a significant increase in the volume of urine.

TABLE III.

Experiment 10. — Injection fluid:  $\frac{1}{2}$  molecular sodium chloride.

Time.	Volume of salt solution injected in last interval of time.	Volume of urine excreted in last interval of time.	Remarks.
a.m. 10.30-10.35	c.c. ..	c.c. ..	Rabbit of 2360 gms. No sugar in urine.
....	..	..	Carotid exposed under ether. Animal taken from board. Quick recovery from the influence of the anæsthetic.
11.15	..	0	<i>Injection into left carotid.</i>
11.17	15	8	No sugar in urine. Respiration rapid.
11.22	25	1	" " " " shallow.
11.24	10	0	" " " " "
11.28	25	0	" " " " "
11.31	25	5	" " " " poor.
11.35	25	6	" " " <i>Complete anæsthesia. (?)</i>
....	..	..	Respiration very deep and slow.
11.40	25	20	Trace of sugar in urine.
....	..	..	<i>Injection stopped.</i>
11.42	..	4	<i>Marked dyspnœa.</i> Large quantity of sugar in urine.
11.48	..	12	Blood drawn for sugar analysis.
....	..	..	Sugar content of blood = 0.269 per cent.

Why is the sugar content of the blood increased when sodium chloride solutions are introduced into the cerebral arterial circulation? The protocols point out that the introduction of the salt solution had a rapid and significant influence upon the respiration. After the injection of fifteen cubic centimetres of solution the breathing was rapid and shallow. When one hundred cubic centimetres had been injected, the animal was limp and did not respond to cutaneous stimulation. This condition of anæsthesia (!) maintained for a time gradually led to labored breathing and dyspnœa. Similar

manifestations were noted in every experiment in which the salt solution was injected into the carotid. In dogs, dyspnœa<sup>1</sup> inevitably leads to hyperglycæmia, and at times to glycosuria; but glycosuria is not so readily produced in these animals as in rabbits. The rabbit kidney seems much more permeable for sugar.<sup>2</sup> Since dyspnœa, or at least marked changes in the manner and rate of respiration, are regularly produced by injections of salt solutions into the cerebral arterial circulation, and since dyspnœa is effective in increasing the sugar content of the blood, it is reasonable to assume that interference with the respiratory processes induced by the sodium chloride is responsible for the hyperglycæmia observed when sodium chloride is injected into the carotid artery.

#### THE GLYCOSURIA PRODUCED BY MAGNESIUM SULPHATE.

Meltzer and Auer<sup>3</sup> have published the details of an experiment in which, after the subcutaneous injection of magnesium sulphate, the appearance in the urine of a substance which reduced Fehling's solution was noted. We have attempted to learn whether the glycosuria produced by intravenous injections of magnesium sulphate was accompanied by hyperglycæmia. An example is given in Table IV.

Here, also, hyperglycæmia was noted, together with an entire absence of polyuria, even though the injection was made into the ear vein. The quantity of magnesium sulphate required to call forth glycosuria is very small compared with sodium chloride in equimolecular solutions. It is probable that the effects produced by magnesium sulphate are identical with those brought about by sodium chloride introduced into the cerebral arterial circulation.

#### SUMMARY.

When sodium chloride is injected into the venous circulation of the rabbit, polyuria and glycosuria are in evidence, probably as a result of an increased permeability of the kidney. The permeability of the

<sup>1</sup> UNDERHILL: *Loc. cit.*

<sup>2</sup> A personal communication from Professor VON NOORDEN that the experiences in his laboratory lead to similar conclusions (F. P. U.).

<sup>3</sup> MELTZER and AUER: This journal, 1905, xiv, p. 366; see Experiment 5, p. 371.

kidney may be decreased by injection of a mixture of sodium chloride and calcium chloride, as indicated by the temporary decreased flow of urine and diminished or inhibited excretion of sugar. Further

TABLE IV.

Experiment 8. — Injection fluid:  $\frac{1}{2}$  molecular magnesium sulphate.

Time.	Volume of salt solution injected in last interval of time.	Volume of urine excreted in last interval of time.	Remarks.
p.m.	c.c.	c.c.	
....	..	..	Rabbit of 1760 gms. No sugar in urine.
3.25	..	..	<i>Injection begun.</i>
3.29	3	6	Trace of sugar in urine.
3.34	1	6	“ “ “
3.38	2	0	<i>Anaesthesia!</i>
3.46	3	5	Trace of sugar in urine. Breathing labored.
3.54	3	0	
3.59	2	..	Breathing almost stopped.
4.10	3	7	Large quantity of sugar in urine.
4.16	1	9	“ “ “ “
....	..	..	<i>Pronounced dyspnoea.</i>
4.20	..	..	Blood drawn for sugar analysis.
....	..	..	Sugar content of blood = 0.320 per cent.

evidence that this form of glycosuria is of renal origin is furnished by the observation that during the appearance of sugar in the urine hypoglycæmia is noted, whereas the sugar content of the blood becomes normal, or hyperglycæmia obtains, when the excretion of sugar in the urine is inhibited by injection of a mixture of sodium chloride and calcium chloride.

Injection of sodium chloride into the cerebral arterial circulation induces glycosuria with no polyuria, but with an accompanying hyperglycæmia. The increased content of sugar in the blood may be referred to disturbances of respiratory processes, dyspnoea, provoked by the introduction of sodium chloride.



The mechanism controlling the glycosuria produced by injection of sodium chloride into the circulation of the rabbit is therefore dependent upon the mode of introducing the salt.

Injections of magnesium sulphate into the circulation cause the appearance of sugar in the urine without polyuria and with hyperglycæmia. The mechanism involved may be attributed to the dyspnœa induced.

## THE PROPORTION OF GLUTAMINIC ACID YIELDED BY VARIOUS VEGETABLE PROTEINS WHEN DECOMPOSED BY BOILING WITH HYDROCHLORIC ACID.<sup>1</sup>

By THOMAS B. OSBORNE AND RALPH D. GILBERT.

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OF the many substances forming the complicated mixture which results from boiling proteins with strong hydrochloric acid, glutaminic acid is the one that can be separated most easily and completely in a state of purity.

Quantitative determinations of this acid, therefore, have much value in showing structural differences between the various protein bodies.

We have consequently found, as accurately as possible, the amount of glutaminic acid yielded by a large number of carefully purified vegetable proteins. Although it is impossible to prove the completeness of this separation, it seems to us to be so nearly quantitative that the results may be taken as representing very nearly the true proportion in which this amino acid is yielded by the protein. This opinion is based on the close agreement between the results obtained by different investigators working on the same protein, and by the fact that the ester method, involving several separate determinations of the glutaminic acid, when carefully conducted, gives the same result as that obtained by the direct method here employed. For example, one of us<sup>2</sup> found 37.3 per cent as a maximum of glutaminic acid yielded by gliadin. Abderhalden and Samuely<sup>3</sup> have since confirmed this result by finding 36.50 per cent.

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> OSBORNE and HARRIS: This journal, 1905, xiii, p. 35.

<sup>3</sup> ABDERHALDEN and SAMUELY: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 194.

In applying Fischer's ester method to the determination of other decomposition products of this protein, a part of the glutaminic acid was obtained from the mixture of the products of hydrolysis by the direct method employed in making the determinations described in this paper, and the remainder was separated in several portions after esterification of the remaining acids and distillation of the esters. The sum of all these portions was equal to 35.9 per cent of glutaminic acid,—a result in such close accord with the figures given above that we have little doubt that it nearly represents the total quantity of glutaminic acid yielded by this protein.

Abderhalden and Samuely<sup>1</sup> have reached the same conclusion after considerable experience with the two methods.

In rare cases we have found it very difficult to bring the glutaminic acid hydrochloride to separate from the original mixture of decomposition products, and it is probable that for these proteins the results obtained have been somewhat too low. These cases are especially noted in the following pages, and we are now engaged in trying to determine whether higher results can be obtained by the ester method. The results of our investigations show a greater difference in the proportion of glutaminic acid yielded by the different seed proteins than has been found heretofore for any other decomposition product of the proteins, with the exception of glycocoll, whose higher limit in the albuminoid fibroin closely approaches, and whose lower limit in many animal and some seed proteins falls below, those of glutaminic acid.

It was also found that glutaminic acid was yielded by *all* of the many seed proteins examined, and is therefore as constant and important a constituent as leucine, over which, in some, it greatly predominates. It was further found that glutaminic acid is yielded in larger proportion by most of these seed proteins than by any of those of animal origin.

None of the large number of animal or vegetable proteins so far examined by suitable methods has failed to yield glutaminic acid, although the amount obtained from some of them was extremely small. We have also found that those proteins which yielded the largest proportion of glutaminic acid in nearly all cases yielded correspondingly large proportions of ammonia, although no strict ratio between the two was found. The following table gives examples of this relation:

<sup>1</sup> ABDERHALDEN and SAMUELY: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 196.

	Glutaminic acid.	N as NH <sub>3</sub> .
Gliadin . . . . .	37.0 per cent	4.20 per cent
Hordein . . . . .	36.0 " "	4.01 " "
Conglutin B. . . . .	30.0 " "	2.65 " "
Glutenin . . . . .	23.4 " "	3.30 " "
Amandin . . . . .	23.0 " "	3.05 " "

Proteins yielding between one and two per cent of nitrogen as ammonia yielded, in general, larger proportions of glutaminic acid, together with a larger proportion of nitrogen as ammonia, although there were many marked exceptions.

It is quite possible that the dibasic character of glutaminic acid has a close connection with this larger proportion of nitrogen split off as ammonia, for one carboxyl group may be bound in the protein molecule by a polypeptide union, while the other is united with NH<sub>2</sub> as an amide. The occurrence of much glutamine and asparagine in the juices of growing plants is in harmony with this supposition. It is, however, too soon to draw conclusions of this sort, as we must have more evidence, especially in regard to the quantity of aspartic, or other dibasic acids, which these proteins yield, before these relations can be properly considered.

It is further interesting to note that among the proteins largely used for human food a very great difference in the yield of glutaminic acid was found; thus gliadin, which forms about one-half the protein substance of wheat and rye, and hordein, forming one-half that of barley, yielded about 37 per cent of glutaminic acid, while cows' milk casein yielded 10.77, ovalbumin from egg white 9 per cent, and conalbumin from egg white 7 per cent. What relation such a difference can bear to the relative nutritive value of these proteins in health or disease is a problem deserving future study.

The method employed for determining the glutaminic acid was essentially that of Hlasiwitz and Habermann.<sup>1</sup> The protein substance, with a few exceptions, especially noted, was treated with an equal *weight* of water, an equal *volume* of concentrated hydrochloric acid added, the mixture heated on the water-bath until it ceased to froth, and then boiled in an oil-bath at 115°-120° for fifteen hours. The resulting solution, which no longer gave a biuret reaction, was then concentrated to about two-thirds its volume and saturated, ice-cold, with gaseous hydrochloric acid. If, on standing for several

<sup>1</sup> HLASIWITZ and HABERMANN: *Annalen der Chemie*, 1873, clxix, p. 150.

days at 0°, after seeding with a few fragments of crystals of glutaminic acid hydrochloride, no considerable quantity of crystals separated, the solution was further concentrated and the treatment repeated. It rarely happened that the second concentration was necessary. The crystalline product that separated was sucked as dry as possible on an asbestos filter, and washed with ice-cold alcoholic hydrochloric acid. The crystals were dissolved in water, the solution decolorized by boiling with animal charcoal, and then freed from ammonia by boiling with an excess of baryta, until all the ammonia was expelled. After removing the baryta with an equivalent quantity of sulphuric acid, the solution was concentrated until crystals began to separate, when it was cooled and allowed to stand on ice for some time. The crystalline product was then sucked as dry as possible and washed with ice-cold hydrochloric acid. The mother liquor and washings were concentrated, and by a repetition of the above treatment a second small crop of crystals was secured.

The glutaminic acid hydrochloride thus obtained was free from color, well crystallized, and, when once recrystallized, was entirely pure. We later found that in almost every case the glutaminic acid as weighed, and before recrystallizing, was so pure that it showed the correct melting-point and composition.

In order to determine the agreement between different operators with this method, we applied it to a sample of Merck's "pure milk casein" in order to compare the result of our determination with that obtained by Emil Fischer, who used this method for the same substance under somewhat different conditions but with essentially the same result. One hundred gm. of the air-dry casein, equal to 90.11 gm. dried at 110°, were boiled with 100 c.c. of concentrated hydrochloric acid for about twelve hours, and then treated in the manner described. The total glutaminic acid hydrochloride weighed 12.01 gm., equal to 9.72 gm. of glutaminic acid, or 10.77 per cent.

From 500 gm. of Merck's casein Fischer<sup>1</sup> got 50 gm. of glutaminic acid, equal to 10 per cent, and Abderhalden<sup>2</sup> later found the percentage as 10.7.

#### PROTEINS OF THE CEREALS.

**Alcohol soluble.**—The seeds of the cereals, so far as these have been examined, are sharply differentiated from those of other plants

<sup>1</sup> FISCHER: *Zeitschrift für physiologische Chemie*, 1901, xxxiii, p. 151.

<sup>2</sup> ABDERHALDEN: *Ibid.*, 1905, xlv, p. 23.

by the presence of a large proportion of protein matter soluble in relatively strong alcohol which is either wholly insoluble or only slightly so in pure water or neutral saline solutions. At least one-half of the protein substance of the seeds of wheat, rye, barley, and maize consists of alcohol soluble protein, and from all these proteins the proportion of glutaminic acid obtained was relatively large.

**Wheat, Gliadin.**—Gliadin, the alcohol soluble protein of wheat, has been the subject of a special study in this laboratory, the results of which have already been published.<sup>1</sup> In this paper the data at present available for determining the individuality of this protein are given, and also the proportion of glutaminic acid which various preparations of this protein yielded. Four determinations of this acid, in as many different preparations, when decomposed with boiling hydrochloric acid gave:

1. 37.00 per cent.
2. 37.33 “ “
3. 34.00 “ “
4. 35.50 “ “

Although the difference between the extremes of these figures is considerable, we think they agree about as closely as the nature of the process of separation will permit.

Abderhalden and Samuely<sup>2</sup> have recently obtained 31.5 per cent of glutaminic acid from wheat gliadin, but as their preparation of this protein yielded 12 per cent of humus (!) while ours yielded no weighable amount, it seems probable that this lower result was due to the character of the product which they examined, with respect to the preparation of which they give no data.<sup>3</sup>

**Rye, Gliadin.**—The seeds of rye yield an alcohol soluble protein which the writer has extensively studied, and found to be in all respects so similar to gliadin that there is at present no reason for doubting the identity of this protein from these two seeds.<sup>4</sup>

In agreement with this experience we have likewise found that

<sup>1</sup> OSBORNE and HARRIS: This journal, 1905, xiii, p. 36.

<sup>2</sup> ABDERHALDEN and SAMUELY: Zeitschrift für physiologische Chemie, 1905, xlv, p. 276.

<sup>3</sup> Very recently ABDERHALDEN and SAMUELY (*Ibid.*, xlv, p. 194) have revised their figures for glutaminic acid from gliadin, and now state the percentage to be 36.5.

<sup>4</sup> OSBORNE: Journal American Chemical Society, 1895, xvii, 429; *Ibid.*, 1903, xxv, p. 323.



gliadin from rye yields about the same proportion of glutaminic acid as gliadin from wheat.

The substance used for this determination was a part of the pure preparation remaining from our former investigation. Thirty gm. of the air-dry material, equal to 28.5 gm. dried at 110°, were mixed with 30 c.c. of water, 30 c.c. of pure concentrated hydrochloric acid added, and boiled for fifteen hours. By the method already described, we obtained 12.04 gm. of glutaminic acid hydrochloride, equivalent to 9.57 gm. of glutaminic acid, or 33.81 per cent. This product, when once recrystallized, melted at 197°, and gave the following results on analysis:

*Carbon and hydrogen.*—0.3294 gm. substance gave 0.4019 gm. CO<sub>2</sub> and 0.1763 gm. H<sub>2</sub>O = C 33.28 and H 5.95 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.<sup>1</sup>

*Nitrogen.*—0.7250 gm. substance gave NH<sub>3</sub> = 5.51 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.60 per cent. Calculated 7.64 per cent.

*Barley, Hordein.*—Hordein, constituting about one-half the protein substance of barley seeds, is very similar in properties to gliadin, but has such a distinctly different composition that there is no doubt that it is a different substance.<sup>2</sup> Hordein, like gliadin, is freely soluble in relatively strong alcohol, and also yields, when decomposed by strong acids, the same relatively large proportion of nitrogen as ammonia. It has likewise been found to yield the same large proportion of glutaminic acid, as the following determination shows.

Thirty gm., equal to 28.2 gm. dried at 110°, of a very carefully purified preparation of hordein were decomposed by boiling with concentrated hydrochloric acid for ten hours. The solution, which no longer showed a biuret reaction, was saturated, ice-cold, with gaseous hydrochloric acid, and after remaining on ice for three days, the very abundant crystalline product which had separated was filtered out, dissolved in water, and treated with an excess of barium hydroxide. Carbonic acid was then passed through the solution until most of the free ammonia had been expelled and the barium hydroxide had been converted into the carbonate. The latter was then filtered out,

<sup>1</sup> The somewhat too high result for C and H in this, as well as in many of the analyses which follow, is unquestionably due to the well-known difficulty experienced in holding back *all* of the large proportion of hydrochloric acid present in this compound.

<sup>2</sup> Cf. OSBORNE: Journal American Chemical Society, 1895, xvii, p. 539.

and the small amount of barium remaining in the solution was quantitatively removed by sulphuric acid. An abundant excess of hydrochloric acid was then added to the solution, and the latter concentrated on the water bath until the glutaminic acid hydrochloride began to separate. After cooling for some time, the latter was filtered out, washed with ice-cold alcoholic hydrochloric acid, dried to constant weight, and found to weigh 12.93 gm. The mother liquor by further concentration yielded 0.48 gm. more, making a total of 13.41 gm. As there was some doubt about the complete removal of ammonia by the cold treatment employed in this case, a part of the crystalline product was distilled with magnesia, and ammonia equivalent to 0.6167 gm. of ammonium chloride was found. Deducting this, we have 12.79 gm., equal to 10.25 gm. of glutaminic acid, or 36.35 per cent of the hordein.

The above product when once recrystallized melted at  $197^{\circ}$ , and was analyzed with the following results:

*Carbon and hydrogen.* — 0.2766 gm. of substance gave 0.3370 gm.  $\text{CO}_2$  and 0.1510 gm.  $\text{H}_2\text{O}$  = C 33.23; H 6.07 per cent. Calculated for  $\text{C}_5\text{H}_9\text{NO}_4\text{HCl}$ : C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.6149 gm. of substance gave  $\text{NH}_3$  = 4.62 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.66 per cent. Calculated 7.64 per cent.

**Maize, Zein.** — The principal protein of maize is zein, which is freely soluble in very strong alcohol. The properties and composition of this peculiar protein are extensively described in papers by Chittenden and Osborne,<sup>1</sup> and by the writer.<sup>2</sup>

The zein used for this determination was prepared by extraction with alcohol, and purified by alternately pouring its alcoholic solution into water and absolute alcohol, containing much ether.

Of this preparation 36.3 gm., equal to 32.44 gm. dried at  $110^{\circ}$ , were treated in the way just described for hordein, and 6.8275 gm. of glutaminic acid hydrochloride was obtained, which was entirely free from ammonia and contained 7.65 per cent of N; calculated 7.64 per cent. This was equivalent to 5.47 gm. glutaminic acid, or 16.87 per cent. Once recrystallized, it melted at  $197.5^{\circ}$ , and gave the following results on analysis:

<sup>1</sup> CHITTENDEN and OSBORNE: American chemical journal, 1891, xiii, pp. 327, 385; also 1892, xiv, p. 20.

<sup>2</sup> OSBORNE: Journal American Chemical Society, 1897, xix, p. 525.

*Carbon and hydrogen.* — 0.3650 gm. substance gave 0.4441 gm. CO<sub>2</sub> and 0.1950 gm. H<sub>2</sub>O = C 33.18; H 5.94 per cent.

*Nitrogen.* — In substance before recrystallizing. 0.5380 gm. substance gave NH<sub>3</sub> = 4.12 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.65 per cent. Calculated 7.64 per cent.

#### WATER SOLUBLE PROTEINS.

*Wheat, Leucosin.* — The embryo of wheat contains a large proportion of leucosin, a protein soluble in pure water and coagulable by heat, therefore possessing the properties characteristic of an albumin. This is one of the very few seed proteins having such properties that the writer has been able to obtain in considerable quantity from any of the many seeds which he has examined.<sup>1</sup>

The preparation used in this work was extracted from a freshly prepared commercial product that consisted almost wholly of the embryo of wheat, the remainder being bran and endosperm which had not been completely removed in the process of manufacture.

The aqueous extract of this material was strained through fine cloth, and, as the resulting turbid solution could not be filtered within a reasonable time, an equal volume of a saturated solution of pure ammonium sulphate was added. The precipitate produced was filtered out, freed carefully from mother liquor, and suspended in a relatively small quantity of water. The solution which resulted was then filtered *perfectly* clear, and coagulated at 65° by heating in a water bath at 70°. The coagulum was then washed very thoroughly with hot water and dehydrated by absolute alcohol. Of this product 100 gm. air dry, equal to 85.78 gm. dried at 110°, were hydrolyzed, and the glutaminic acid separated in the way described in the earlier part of this paper.

The glutaminic acid hydrochloride obtained weighed 6.13 gm., equal to 4.91 gm. of the free acid, or 5.72 per cent.

After once recrystallizing, the glutaminic acid hydrochloride when dried at 100° melted at 197.5°, and gave the following results on analysis:

<sup>1</sup> Cf. OSBORNE and VOORHEES: American chemical journal, 1893, xv, p. 392; also OSBORNE and CAMPBELL, Journal American Chemical Society, 1900, xxii, 1-379.

*Carbon and hydrogen.*—0.3191 gm. substance gave 0.3870 gm. CO<sub>2</sub> and 0.1665 gm. H<sub>2</sub>O = C 33.08; H 5.80 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.*—0.5482 gm. substance gave NH<sub>3</sub> = 4.17 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.61 per cent. Calculated 7.64 per cent.

Great difficulty was found in bringing this glutaminic acid hydrochloride to separate, and it is possible that the result here given is somewhat too low. We are now engaged in esterifying this protein, and it is possible that more glutaminic acid may be obtained from the esters.

#### ALKALI SOLUBLE PROTEINS.

**Wheat, *Glutenin.***—That part of wheat gluten which is not soluble in alcohol of 70 per cent consists chiefly of a protein insoluble in water and saline solutions but readily soluble in dilute alkali or acid, from which solution it can be precipitated by neutralization without any detectable change. This protein, which Ratthausen called gluten casein, has been extensively described by the writer under the name of glutenin.<sup>1</sup> The material used for our determinations of glutaminic acid was prepared in large quantity from carefully washed gluten. This was ground while moist in a drug press, which reduced it to comparatively small pieces, and then extracted with alcohol as long as any considerable amount of gliadin was removed from it. The residue was then thoroughly dried in the air and ground to a fine powder. This powder was next extracted for a long time with absolute alcohol and then with ether, until all the lecithin and fat had been removed, when it was freed from alcohol and ether at the room temperature, and then dissolved by adding a just sufficient quantity of 0.2 per cent solution of potassium hydroxide. On shaking for a short time nearly all of the powder thus prepared dissolved, and the resulting solution was quickly filtered *perfectly clear*. The clear solution was then neutralized with very dilute hydrochloric acid, and the voluminous precipitate that resulted was exhausted with 70 per cent alcohol so long as *any* gliadin could be removed. This required very long continued and frequently repeated treatment. The product that remained was then thoroughly dehydrated with absolute alcohol and dried over sulphuric acid. It formed a white, dusty powder, and represented the purest preparation of

<sup>1</sup> Cf. OSBORNE and VOORHEES: American chemical journal, 1893, xv, p. 392.

glutenin that we know how to make. Few proteins present more difficulties in their preparation than does glutenin, for it is absolutely essential to its purification that its solution be filtered perfectly clear, and this, so far as we have found, can only be done on a large scale by the procedure described above. The complete removal of gliadin can be effected only by using very large quantities of alcohol and continuing the extraction for many days.

One hundred gm. of glutenin, air dry, equal to 93.21 gm. dried at 110°, yielded 26.21 gm. of glutaminic acid hydrochloride, equal to 20.97 gm. of glutaminic acid, or 22.53 per cent. After once recrystallizing, this melted at 197° and dried at 100° gave the following results on analysis:

*Carbon and hydrogen.* — 0.2845 gm. of substance gave 0.3433 gm. CO<sub>2</sub> and 0.1505 gm. H<sub>2</sub>O = C 32.91; H 5.87 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.5911 gm. substance gave NH<sub>3</sub> = 4.53 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.66 per cent. Calculated 7.64 per cent.

A second determination was made by hydrolyzing 50 gm. of a different preparation of glutenin, equal to 48.9425 gm. dried at 110°. This gave 14.3068 gm. of glutaminic acid hydrochloride, equivalent to 11.464 gm. glutaminic acid, or 23.42 per cent of glutaminic acid from glutenin.

Without recrystallizing, the hydrochloride, as weighed, melted at 199°, and contained 7.62 per cent of nitrogen.

*Nitrogen.* — 0.7575 gm. substance gave NH<sub>3</sub> = 5.77 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.62 per cent. Calculated 7.64 per cent.

Kutscher<sup>1</sup> obtained 9 per cent of glutaminic acid from a preparation of glutenin, using a different method from that employed by us. This result, like those which he obtained with the same method applied to the alcohol-soluble protein from wheat, is far lower than ours, and certainly does not even approximate to the true content of this protein in glutaminic acid.

#### PROTEINS OF THE LEGUMES.

**Kidney bean, *Phaseolin.*** — The kidney or white bean (*Phaseolus vulgaris*) contains a large proportion of protein, most of which con-

<sup>1</sup> KUTSCHER: *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 126.



sists of a globulin which one of us has described under the name of phaseolin.<sup>1</sup>

One hundred gm. of a very carefully purified preparation of phaseolin, equivalent to 89.83 gm. dried at 110°, yielded 13.8246 gm. of glutaminic acid hydrochloride, equal to 11.0777 gm. of glutaminic acid, or 12.33 per cent. This glutaminic acid hydrochloride, without recrystallizing, melted at 198°, and gave the following results on analysis:

*Carbon and hydrogen.* — 0.2517 gm. of substance gave 0.3011 gm. CO<sub>2</sub> and 0.1367 gm. H<sub>2</sub>O = C 32.62; H 6.03 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.3477 gm. substance gave NH<sub>3</sub> = 2.66 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.65 per cent. Calculated 7.64 per cent.

*Vetches, beans, peas, and lentils, Legumin.* — The seeds of the vetch (*Vicia sativa*), the horse bean (*Vicia faba*), the lentil (*Ervum lens*), and the garden pea (*Pisum sativum*) contain a relatively large proportion of protein which, in a state of more or less purity, has been long known under the name of legumin. This protein has been very carefully studied by one of us,<sup>2</sup> and thoroughly purified preparations from each of these seeds were found entirely alike in respect to all the properties that could at that time be compared.

Unfortunately this comparison cannot at present be extended to the amount of glutaminic acid yielded by preparations from these different seeds, for only one preparation, that from the vetch, is now available.

Of this legumin from the vetch 21.3357 gm. of a very carefully purified preparation gave 4.391 gm. of glutaminic acid hydrochloride, equal to 3.5185 gm. glutaminic acid, or 16.48 per cent of the legumin.

This glutaminic acid hydrochloride, as weighed in the above determination, melted at 198°, and gave the following results on analysis:

*Carbon and hydrogen.* — 0.3661 gm. substance gave 0.4375 gm. CO<sub>2</sub> and 0.1833 gm. H<sub>2</sub>O = C 32.59; H 5.56 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.4138 gm. substance gave NH<sub>3</sub> = 3.24 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.83 per cent. Calculated 7.64 per cent.

<sup>1</sup> OSBORNE: Journal American Chemical Society, 1894, xvi, pp. 633, 703, 757.

<sup>2</sup> OSBORNE and CAMPBELL: *Ibid.*, 1896, xviii, p. 583; also 1898, xxi, pp. 348, 362, 393, 406, 410.



The cow pea, *Vignin*. — The cow pea (*Vigna catjang*) contains a large amount of protein which closely resembles legumin, but differs from the latter in composition to such an extent as to leave no doubt that the two are distinctly different substances. This protein was described by the writer under the name of vignin.<sup>1</sup>

It was later found<sup>2</sup> that legumin contained considerably more basic nitrogen than vignin, but this difference is not supported by our determinations of glutaminic acid, for we have obtained the same amount from each.

Thirty gm. of one of our former pure preparations of vignin, equivalent to 29.19 gm. dried at 110°, yielded 6.1646 gm. of glutaminic acid hydrochloride, equal to 4.932 gm. of glutaminic acid, or 16.89 per cent.

After recrystallizing once, this melted at 196° and gave the following results on analysis:

*Carbon and hydrogen.* — 0.3344 gm. substance gave 0.4062 gm. CO<sub>2</sub> and 0.1764 gm. H<sub>2</sub>O = C 33.13 and H 5.86 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.7476 gm. substance gave NH<sub>3</sub> = 5.68 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.59 per cent. Calculated 7.64 per cent.

*Soy bean, Glycinin.* — One of us has investigated the proteins of two varieties of the soy bean (*Glycine hispida*), one the yellow soy bean and the other a Japanese variety known as the Kiyusuki daidzu.<sup>3</sup>

The substance constituting by far the greater part of the protein in these seeds was named glycinin, and a strict comparison of preparations from the two seeds revealed no differences.

Of the glycinin from the yellow soy bean 24.5 gm., equal to 22.23 gm. dried at 110°, yielded 5.407 gm. of glutaminic acid hydrochloride, equal to 4.327 gm. of glutaminic acid, or 19.46 per cent.

When once recrystallized, this melted at 196.5°, and gave, on analysis, the following results:

*Carbon and hydrogen.* — 0.3191 gm. substance gave 0.3876 gm. CO<sub>2</sub> and 0.1676 gm. H<sub>2</sub>O = C 33.13; H 5.84 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.4380 gm. substance gave NH<sub>3</sub> = 3.36 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.67 per cent. Calculated 7.64 per cent.

<sup>1</sup> OSBORNE and CAMPBELL: *Journal American Chemical Society*, 1897, xix, p. 494.

<sup>2</sup> OSBORNE and HARRIS: *Ibid.*, 1903, xxv, p. 323.

<sup>3</sup> OSBORNE and CAMPBELL: *Ibid.*, 1898, xx, p. 419.

Of the glycinin from the Japanese bean (Kiyusuki daidzu) 50.1 gm., equal to 47.77 gm. dried at 110°, yielded 10.68 gm. of glutaminic acid hydrochloride, equal to 8.5579 gm. glutaminic acid, or 17.92 per cent.

Once recrystallized, this melted at 197°, and gave on analysis the following results:

*Carbon and hydrogen.*—0.3509 gm. substance gave 0.4286 gm. CO<sub>2</sub> and 0.1840 gm. H<sub>2</sub>O = C 33.28; H 5.82 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.*—0.5731 gm. substance gave NH<sub>3</sub> = 4.41 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.69 per cent. Calculated 7.64 per cent.

Although the amount of glutaminic acid obtained from these two preparations was not exactly the same, nevertheless we consider the difference to be due to the unavoidable errors of the process of separation, and that these determinations afford no ground for assuming that actual differences exist between these proteins of the two seeds.

*Lupine, Conglutins.*—Previous investigations by one of us have shown that the greater part of the protein of the yellow lupine seeds consists of a mixture of two globulins which, though in many respects much alike, present such distinct differences that we have no doubt that two different globulins occur. In the seeds of the blue lupine no evidence of a second globulin was obtained, practically the whole of the protein consisting, so far as could be determined, of one substance, which agreed in properties and composition so closely with one of those found in the yellow lupine that the identity of the two seemed probable.

Our present determinations of glutaminic acid from these globulins has made a further comparison possible.<sup>1</sup>

Yellow lupine seeds yield nearly all their protein on extraction with sodium chloride solutions, and this can be separated, by fractional precipitation with ammonium sulphate, into two parts of distinctly different composition.

The substances used for these determinations of glutaminic acid were parts of the fractions thrown down by ammonium sulphate and described under the same designations by Osborne and Harris.<sup>2</sup>

<sup>1</sup> Cf. OSBORNE and CAMPBELL: *Journal American Chemical Society*, 1897, xix, p. 454; OSBORNE: *Ibid.*, 1902, xiv, p. 140; OSBORNE and HARRIS: *Ibid.*, 1903, xxv, p. 323; *Ibid.*, This journal, 1905, xiii, p. 436.

<sup>2</sup> OSBORNE and HARRIS: This journal, 1905, xiii, p. 436.

Of fraction No. 1, conglutin A, thrown down between  $\frac{0}{10}$  and  $\frac{5}{10}$  saturation with ammonium sulphate, a quantity equal to 17.0601 gm. dried at  $110^\circ$  yielded 4.4594 gm. of glutaminic acid hydrochloride, equal to 3.5733 gm. glutaminic acid, or 20.96 per cent.

This substance, as weighed, melted at  $198^\circ$ .

Of fraction No. 2, conglutin A, thrown down between  $\frac{5}{10}$  and  $\frac{6}{10}$  saturation with ammonium sulphate, a quantity equal to 27.8790 gm. dried at  $110^\circ$  gave 6.5875 gm. glutaminic acid hydrochloride, equal to 5.2786 gm. glutaminic acid, or 18.94 per cent.

This substance, as weighed, melted at  $198^\circ$ .

*Nitrogen.*—0.6107 gm. substance gave  $\text{NH}_3 = 4.66$  c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.63 per cent. Calculated 7.64 per cent.

Of the fraction No. 4, thrown down between  $\frac{7}{10}$  and  $\frac{10}{10}$  saturation with ammonium sulphate, conglutin B, we decomposed 33 gm., equal to 30.22 gm. dried at  $110^\circ$ , and obtained 11.3347 gm. glutaminic acid hydrochloride, equal to 9.0825 gm. glutaminic acid, or 30.05 per cent. When once recrystallized, this melted at  $195.5^\circ$ , and gave the following results when analyzed:

*Carbon and hydrogen.*—0.3514 gm. substance gave 0.4289 gm.  $\text{CO}_2$  and 0.1825 gm.  $\text{H}_2\text{O} = \text{C } 33.29$ ;  $\text{H } 5.77$  per cent. Calculated for  $\text{C}_5\text{H}_9\text{NO}_4\text{HCl}$ :  $\text{C } 32.67$ ;  $\text{H } 5.50$  per cent.

*Nitrogen.*—0.8524 gm. substance gave  $\text{NH}_3 = 6.50$  c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.62 per cent. Calculated 7.64 per cent.

The great difference in the proportion of glutaminic acid yielded by these two fractions leaves no room for doubt that fundamental differences in the structure of their molecules exist. It is also worth noting that in harmony with our experience with most of the other proteins, conglutin B yielded more nitrogen as ammonia than conglutin A; *i. e.*, 2.65, and 2.12 per cent respectively. The globulin from the blue lupine has shown no evidence of being a mixture when subjected to fractional precipitation from sodium chloride solutions. We have had no opportunity to apply to this substance a rigid fractionation with ammonium sulphate, but such experiments as we have tried indicate that our preparations contain very little, if any, substance corresponding to conglutin B.

29.6 gm. of pure conglutin (from the blue lupine) equal to 26.64 gm. dried at  $110^\circ$ , yielded 7.6451 gm. of glutaminic acid hydro-

chloride, equal to 6.126 gm. glutaminic acid, or 23.00 per cent. Once recrystallized, this melted at 196.5°, and had the following composition :

*Carbon and hydrogen.*—0.3332 gm. substance gave 0.4055 gm. CO<sub>2</sub> and 0.1728 gm. H<sub>2</sub>O = C 33.19; H 5.76 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.677; H 5.50 per cent.

*Nitrogen.*—0.5151 gm. substance gave NH<sub>3</sub> = 3.95 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.67 per cent. Calculated 7.64 per cent.

This result is so little higher than that given by conglutin A that we have no reason for considering the two to be different proteins.

Since these determinations were made, Abderhalden and Herrick<sup>1</sup> have published a partial analysis of "Conglutin from the lupine seed." These investigators do not give the variety of lupine employed, and make no claim to the chemical individuality of their product, which was extracted from the seed by dilute alkali, according to Ritthausen's method. From their preparation they obtained only 6.5 per cent of glutaminic acid, but as this was separated by the ester process, it was not regarded as a quantitative determination.

A comparison between their results and ours obviously cannot be made.

#### OIL SEEDS.

The proteins from the various oil seeds here described bear a certain general resemblance to each other in solubility, reactions, and composition. They all contain a large proportion of nitrogen, 18 to 19 per cent, and are among the richest of the proteins in basic nitrogen. They are here treated together for the sake of convenience, and not because any known relation exists between them. The present investigation shows a wide difference in the proportion of glutaminic acid which the different individuals yield.

**The almond, *Amandin.***—The protein substance of the almond is composed almost wholly of a globulin which contains 19 per cent of nitrogen and yields over 3 per cent of nitrogen as ammonia, thus making it the richest in amide nitrogen of any of the globulins known.<sup>2</sup> It has likewise been found to yield more glutaminic acid than any of the proteins of the oil seeds yet examined.

<sup>1</sup> ABDERHALDEN and HERRICK: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 479.

<sup>2</sup> Cf. OSBORNE and CAMPBELL: *Journal American Chemical Society*, 1896, xviii, p. 609; OSBORNE and HARRIS: *Ibid.*, 1903, xxv, p. 323.

Forty-five gm. of air-dry amandin, equivalent to 40.68 gm. dried at 110°, yielded 11.75 gm. glutaminic acid hydrochloride, equal to 9.4153 gm. of glutaminic acid, or 23.14 per cent. The substance, as weighed, melted at 196.5°. Unfortunately the material was accidentally destroyed before analytical data were obtained. The purity and identity of the substance weighed, however, are sufficiently established by its melting-point.

**Sunflower seed, Globulin.**—Although pure preparations of this protein cannot be made by any method known to us, owing to the presence of much helianthotannic acid in this seed, we have nevertheless determined the proportion of glutaminic acid in a sample of this globulin which consisted of a mixture of the purer preparations described in a former paper from this laboratory.<sup>1</sup> We consider this material to be so nearly pure that the proportion of glutaminic acid yielded by it represents very nearly its true content.

Of this preparation 45.08 gm., equal to 43.85 gm. dried at 110°, yielded 11.92 gm. of glutaminic acid hydrochloride, equal to 9.5515 gm. of glutaminic acid, or 21.79 per cent. When once recrystallized, this substance melted at 197.5°, and gave the following figures on analysis:

*Carbon and hydrogen.*—0.3224 gm. substance gave 0.3907 gm. CO<sub>2</sub> and 0.1695 gm. H<sub>2</sub>O = C 33.05; H 5.84 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.*—0.8372 gm. substance gave NH<sub>3</sub> = 6.38 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.62 per cent. Calculated 7.64 per cent.

This result is considerably higher than the 13.0 per cent recently given by Abderhalden and Reinbold<sup>2</sup> in a partial analysis of protein from the sunflower seed; but as these investigators give no description of the methods employed in preparing the material which they analyzed, it is impossible for us to comment intelligently on this difference.

**Hazel nut, Corylin.**—Under the name of corylin one of us<sup>3</sup> has described a globulin which forms nearly all of the protein of the hazel nut or filbert.

<sup>1</sup> OSBORNE and CAMPBELL: *Journal American Chemical Society*, 1897, xix, p. 487.

<sup>2</sup> ABDERHALDEN and REINBOLD: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 284.

<sup>3</sup> OSBORNE and CAMPBELL: *Journal American Chemical Society*, 1896, xviii, p. 609.



The preparation employed for this determination of glutaminic acid is a part of that fraction precipitated by ammonium sulphate between  $\frac{4}{10}$  and  $\frac{5}{10}$  saturation, as described in a recent paper from this laboratory.<sup>1</sup>

A quantity of this preparation, equal to 39.6 gm. dried at 110°, yielded 8.87 gm. glutaminic acid hydrochloride, equivalent to 7.1075 gm. of glutaminic acid, or 17.94 per cent. When once recrystallized, this substance melted at 197° and gave the following results on analysis:

*Carbon and hydrogen.* — 0.3292 gm. substance gave 0.3999 gm. CO<sub>2</sub> and 0.1712 gm. H<sub>2</sub>O = C 33.13; H 5.78 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>; C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.4326 gm. substance gave NH<sub>3</sub> = 3.28 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.58 per cent. Calculated 7.64 per cent.

*Castor bean, Globulin.* — The castor bean, *Ricinus communis*, contains a large quantity of a globulin which has been described by the writer in several papers.<sup>2</sup>

This globulin in most respects closely resembles edestin from the hemp seed, and, like the latter, yields very nearly the same amount of glutaminic acid.

Fifty gm. of the air-dry globulin, equal to 43.65 gm. dried at 110°, yielded 7.9 gm. of glutaminic acid hydrochloride, equal to 6.3303 gm. glutaminic acid, or 14.50 per cent.

After once recrystallizing, this substance melted at 197° and was analyzed with the following results:

*Carbon and hydrogen.* — 0.3352 gm. of substance gave 0.3831 gm. CO<sub>2</sub> and 0.1636 gm. H<sub>2</sub>O = C 33.14; H 5.79 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.4504 gm. substance gave NH<sub>3</sub> = 3.46 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.68 per cent. Calculated 7.64 per cent.

*Para or Brazil nut, Excelsin.* — By far the greater part of the protein substance of the Brazil nut (*Bertholetia excelsa*) consists of a globulin which can be obtained in beautiful hexagonal crystals.

One hundred gm. of these crystals, equal to 91.34 gm. dried at

<sup>1</sup> OSBORNE and HARRIS: This journal, 1905, xiii, p. 436.

<sup>2</sup> OSBORNE: American chemical journal, 1892, xiv, p. 662; OSBORNE and CAMPBELL: Journal American Chemical Society, 1897, xix, p. 482; OSBORNE and HARRIS: This journal, 1905, xiv, p. 259.



110°, yielded 14.7545 gm. of glutaminic acid hydrochloride, equal to 11.8228 gm. of glutaminic acid, or 12.94 per cent.

When once recrystallized, this substance melted at 197°, and gave the following results on analysis:

*Carbon and hydrogen.* — I. 0.3857 gm. substance gave 0.4648 gm. CO<sub>2</sub> and 0.2065 gm. H<sub>2</sub>O = C 32.87; H 5.94 per cent.

II. 0.3643 gm. substance gave 0.4400 gm. CO<sub>2</sub> and 0.1894 gm. H<sub>2</sub>O = C 32.91; H 5.77 per cent.

Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.5202 gm. substance gave NH<sub>3</sub> 3.92 = c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.54 per cent. Calculated 7.64 per cent.

**Cotton seed, Globulin.** — A large part of the protein substance of the cotton seed (*Gossypium herbacium*) consists of the globulin employed for this determination.<sup>1</sup>

This globulin was made by exhausting cotton seed meal with water, until the greater part of the coloring matter and other water-soluble substances had been removed, and then extracting the residue with ten per cent sodium chloride solution. The perfectly clear extract was then dialyzed, and the precipitated globulin redissolved in brine, and its solution again precipitated by dialysis. The globulin thus separated was washed thoroughly with water and with alcohol and dried over sulphuric acid.

46.76 gm. of this globulin, dried at 110°, yielded 10.2662 gm. of glutaminic acid hydrochloride, equal to 8.2263 gm. of glutaminic acid, or 17.59 per cent.

This substance melted at 198°, and gave on analysis the following results:

*Carbon and hydrogen.* — 0.4812 gm. substance gave 0.5827 gm. CO<sub>2</sub> and 0.2435 gm. H<sub>2</sub>O = C 33.02; H 5.62 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.* — 0.5691 gm. substance gave NH<sub>3</sub> = 4.35 HCl (1 c.c. HCl = 0.01 gm. N) = 7.64 per cent. Calculated 7.64 per cent.

Abderhalden and Rostoski<sup>2</sup> found in the "edestin from cotton seed" 17.2 per cent of glutaminic acid, with which figure our result

<sup>1</sup> Cf. OSBORNE and VOORHEES: *Journal American Chemical Society*, 1894, xvi, p. 778.

<sup>2</sup> ABDERHALDEN and ROSTOSKI: *Zeitschrift für physiologische Chemie*, 1905, xliv, p. 265.

agrees very closely. In another "less pure" preparation of the protein of this seed they found, as the result of a careful determination, 14.5 per cent of glutaminic acid. Concerning the method employed in making these preparations of this protein, they give no details.

**Squash seed, Globulin.**—The greater part of the protein substance of the squash seed (*Cucurbita maxima*) consists of a globulin which is obtained in octahedral crystals with comparative ease.<sup>1</sup>

Of this globulin 49.6523 gm., equal to 46.7228 gm. dried at 110°, yielded 7.1746 gm. of glutaminic acid hydrochloride, equal to 5.749 gm. of glutaminic acid, or 12.35 per cent.

On once recrystallizing, this substance melted at 197.5°, and was analyzed with the following results :

*Carbon and hydrogen.*—0.3582 gm. of substance gave 0.4331 gm. CO<sub>2</sub> and 0.1893 gm. H<sub>2</sub>O = C 32.98 ; H 5.87 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67 ; H 5.50 per cent.

*Nitrogen.*—0.5588 gm. substance gave NH<sub>3</sub> = 4.2 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.52 per cent. Calculated 7.64 per cent.

**Hemp seed, Edestin.**—A very large part of the protein substance of this seed consists of a globulin, edestin, which on account of the ease with which it can be obtained in octahedral crystals has been the subject of extensive study. The material used for this determination was of great purity, and was prepared according to the method described by the writer.<sup>2</sup>

Of this substance 75 gm., equal to 68.38 gm. dried at 110°, yielded 11.94 gm. of glutaminic acid hydrochloride, equal to 9.57 gm. of the free acid, or 14.0 per cent.

The hydrochloride in the condition in which it was weighed for this determination melted at 198°.

*Nitrogen.*—0.5027 gm. substance gave NH<sub>3</sub> = 3.85 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.66 per cent. Calculated for C<sub>5</sub>H<sub>10</sub>NO<sub>4</sub>Cl: 7.64 per cent.

This result shows that edestin contains much more glutaminic acid than the 6.3 per cent found by Abderhalden by the ester method.<sup>3</sup>

<sup>1</sup> GRÜBLER: *Journal für praktische Chemie*, 1881, xxiii, p. 97; CHITTENDEN and HARTWELL: *Journal of physiology*, 1890, xi, p. 435; OSBORNE: *American chemical journal*, 1892, xiv, p. 662; OSBORNE and HARRIS: *Journal American Chemical Society*, 1903, xxv, p. 323.

<sup>2</sup> OSBORNE: *Journal American Chemical Society*, 1902, xxiv, p. 39; also *Zeitschrift für physiologische Chemie*, 1901, xxxiii, p. 240.

<sup>3</sup> ABDERHALDEN: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 21.

## ANIMAL PROTEINS.

**Albumins from hen's egg, Ovalbumin.**—An extended study made by one of us of the proteins of the egg white<sup>1</sup> showed that at least two distinct albumins were present in this substance. We therefore included in this investigation determinations of the proportion of glutaminic acid yielded by each of these proteins, in order to learn if a difference in the structure of these two albumins could be thus revealed, and also to compare them with the vegetable proteins already examined.

The preparation of ovalbumin which we have used was a part of the purest crystalline fractions described in the paper referred to.

Fifty gm. of this crystallized ovalbumin, equal to 48.1357 gm. dried at 110°, yielded 5.4147 gm. glutaminic acid hydrochloride, equal to 4.3387 gm. glutaminic acid, or 9.01 per cent. Once recrystallized, this melted at 196°, and on analysis gave the following results:

*Carbon and hydrogen.*—0.3278 gm. substance gave 0.3979 gm. CO<sub>2</sub> and 0.1727 gm. H<sub>2</sub>O = C 33.11; H 5.85 per cent. Calculated for C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>HCl: C 32.67; H 5.50 per cent.

*Nitrogen.*—0.3748 gm. substance gave NH<sub>3</sub> = 2.89 c.c. HCl (1 c.c. HCl = 0.01 gm. N) = 7.71 per cent. Calculated 7.64 per cent.

Abderhalden and Pregl<sup>2</sup> have recently published a partial analysis of crystallized ovalbumin in which they give 8 per cent of glutaminic acid,—a result in good agreement with ours, although obtained by the ester method.

*Conalbumin.*—Under the name of conalbumin one of us has described the albumin which occurs in considerable quantity in the white of hens' eggs. The composition and properties of conalbumin are given in a paper from this laboratory,<sup>3</sup> and in a later paper<sup>4</sup> attention is called to the proportion of basic nitrogen yielded by ovalbumin and by conalbumin, which indicates a distinct difference in the constitution of the molecules of these two proteins.

<sup>1</sup> OSBORNE and CAMPBELL: Journal American Chemical Society, 1899, xxi, p. 477; also 1900, xxii, p. 422.

<sup>2</sup> ABDERHALDEN and PREGL: Zeitschrift für physiologische Chemie, 1905, xlv, p. 24.

<sup>3</sup> OSBORNE and CAMPBELL: Journal American Chemical Society, 1900, xxii, p. 422.

<sup>4</sup> OSBORNE and HARRIS: *Ibid.*, 1903, xxv, p. 323.

The preparation used for the determination of glutaminic acid was a part of that used for the determination of basic nitrogen. The method by which this was made is given on page 346 of the paper last referred to. As only a relatively small amount of this protein was available, we were compelled to use but little substance for this determination.

A quantity corresponding to 7.8 gm. of conalbumin, dried at 110°, yielded 0.6805 gm. of glutaminic acid hydrochloride, equal to 0.5452 gm., or 7 per cent of glutaminic acid.

Once recrystallized, this substance melted at 196°, and contained the following amount of nitrogen :

*Nitrogen.* — 0.5364 gm. of substance gave  $\text{NH}_3 = 4.08$  c.c.  $\text{HCl}$  (1 c.c.  $\text{HCl} = 0.01$  gm.  $\text{N}$ ) = 7.61 per cent. Calculated for  $\text{C}_5\text{H}_9\text{NO}_4\text{HCl}$ :  $\text{N} = 7.64$  per cent.

The proportion of glutaminic acid thus found is less than that obtained from ovalbumin, but the difference is not sufficient to warrant the conclusion that there is, in this respect, a distinct difference in structure between these two proteins. This is especially so, in view of the small amount of conalbumin available for this analysis.

The following table gives a summary of the results of the preceding determinations :

PROPORTION OF GLUTAMINIC ACID YIELDED BY VARIOUS PROTEINS.

VEGETABLE PROTEINS.

<i>Cereals. — Proteins soluble in alcohol.</i>	Per cent.
Gladin, wheat . . . . .	37.17
Gladin, rye . . . . .	33.81
Hordein, barley . . . . .	36.35
Zein, maize . . . . .	16.87
<i>Proteins soluble in water.</i>	
Leucosin, wheat . . . . .	5.72
<i>Proteins soluble in alkali.</i>	
Glutenin . . . . .	23.42
<i>Legumes. — Proteins soluble in saline solutions.</i>	
Phaseolin, kidney bean . . . . .	12.33
Legumin, vetch . . . . .	16.48

Legumes ( <i>continued</i> ).	per cent.
Vignin, cow pea . . . . .	16.89
Glycinin, yellow soy bean . . . . .	19.46
Glycinin, Japanese soy bean . . . . .	17.92
Conglutin, A, yellow lupine . . . . .	20.96
Conglutin, B, yellow lupine . . . . .	30.05
Conglutin, blue lupine . . . . .	23.00
<b>Oil Seeds. — <i>Proteins soluble in saline solutions.</i></b>	
Amandin, almonds . . . . .	23.14
Globulin, sunflower . . . . .	21.79
Corylin, hazel nut . . . . .	17.94
Globulin, castor bean . . . . .	14.50
Excelsin, Brazil nut . . . . .	12.94
Globulin, cotton seed . . . . .	17.59
Globulin, squash seed . . . . .	12.35
Edestin, hemp seed . . . . .	14.00

## ANIMAL PROTEINS.

Casein, cows' milk . . . . .	10.77
Ovalbumin, hens' egg . . . . .	9.01
Conalbumin, hens' egg . . . . .	7.00

This table shows that the proteins of the cereals yield much more glutaminic acid than do any of the other groups, for, omitting leucosin, which is present in the wheat kernel only in very small proportion and confined chiefly to the embryo of the seed, the average yield of this acid was 29.5 per cent, while the legumes yielded 19.6 per cent, the oil seeds 16.8 per cent, and the three animal proteins 8.9 per cent.

An examination of the literature up to the time this work was carried to this point showed that such other animal proteins as had been examined had yielded very small proportions of glutaminic acid, most of them less than 3 per cent. It seemed therefore that, as the proteins of the seed endosperm as well as of milk and eggs yielded relatively large quantities of this amino acid, in contrast to the proteins of animal tissues, a distinction could be made between the *food* proteins and the tissue proteins.

With this idea in view we undertook to determine the proportion of glutaminic acid yielded by the muscle tissue of the ox and of the fish (halibut), the results of which have shown, however, that these



tissues yield about the same amount of this acid as did the three animal proteins which we had already examined.

**Beef.**—For this purpose a quantity of lean beef, cut from the rump and freed from adhering fat and connective tissue, was chopped fine, and its nitrogen content found to be 3.68 per cent. Two portions of 300 gm. each were hydrolyzed by boiling I. with 200 cc. of concentrated hydrochloric acid and II. with 100 cc. of the same acid, for fifteen hours.

The glutaminic acid was separated as hydrochloride, as already described.

I. yielded 8.6706 gm. glutaminic acid hydrochloride, equal to 6.9478 gm. glutaminic acid. Assuming all the nitrogen contained in the beef to belong to protein bodies containing 16 per cent of nitrogen, the 300 gm. taken contained 69 gm. of protein, which yielded the above quantity of glutaminic acid, or 10.06 per cent.

II. yielded 9.5541 gm. of glutaminic acid hydrochloride, which melted at 198°. This is equal to 7.6557 gm. glutaminic acid. By the preceding method of calculating, the protein gave 11.09 per cent. As all the nitrogen of the meat is *not* protein nitrogen, and the proteins probably contain somewhat more nitrogen than 16 per cent, the results of this examination show that this muscle tissue of the ox yields somewhat more glutaminic acid than casein and ovalbumin, and about as much as the seed proteins which have given the smallest yields of this acid, leucosin excepted, which yields much less.

**Fish.**—A quantity of the solid muscle tissue from a large halibut was freed from external fat and connective tissue, and found to contain 2.64 per cent of nitrogen. Of this tissue 300 gm. were hydrolyzed by boiling for fifteen hours with 100 c.c. of concentrated hydrochloric acid and 5.4825 gm. of glutaminic acid hydrochloride were obtained from the solution containing the decomposition products. This hydrochloride melted at 198°.

By the same method of calculation as employed for the beef, the protein bodies of this tissue are shown to yield at least 8.88 per cent of glutaminic acid.

Since this work was completed Abderhalden and Samuely<sup>1</sup> have revised the determinations of glutaminic acid in the serum globulin and serum albumin of horse blood, and give as the average of three

<sup>1</sup> ABDERHALDEN and SAMUELY: *Zeitschrift für physiologische Chemie*, 1905, xlvii, p. 194.



careful determinations in each, 8.5 per cent and 7.7 per cent respectively, instead of 2.2 and 1.52 per cent respectively, which Abderhalden formerly gave.<sup>1</sup>

In view of these later figures by Abderhalden, those published earlier by him and his colleagues for other animal proteins cannot be used as a basis for a quantitative comparison, and in justice to those offering them it should be stated that they were given only as minimal quantities.

If these later figures are accepted as representing approximately all of the glutaminic acid in these proteins, it would appear that such animal proteins as have been carefully investigated yield similar proportions of glutaminic acid, namely, about 8-11 per cent, as may be seen from the following table:

GLUTAMINIC ACID YIELDED BY ANIMAL PROTEINS.

Casein . . . . .	10.8 per cent	. .	Osborne and Gilbert.
“ . . . . .	10.0 “ “	. .	Fischer.
“ . . . . .	10.7 “ “	. .	Abderhalden and Pregl.
Ovalbumin . . . . .	9.1 “ “	. .	Osborne and Gilbert.
“ . . . . .	8.0 “ “	. .	Abderhalden.
Seralbumin, horse . . . .	7.7 “ “	. .	Abderhalden and Samuely
Serglobulin, horse . . . .	8.5 “ “	. .	“ “ “
Beef muscle protein . . . .	11.1 “ “	. .	Osborne and Gilbert.
Fish muscle protein . . . .	8.9 “ “	. .	“ “ “

In view of the wide differences in the constitution of the proteins of the different species of seeds, as shown by the determinations of glutaminic acid given in this paper, as well as by the few quantitative determinations of the other decomposition products which have been made, it would seem important to know definitely whether or not similar differences exist between the proteins of the tissues of different species of animals which serve as food for man, for it is possible that in such differences will be found a logical basis for the use of one form of protein rather than another when dealing with nutrition in various pathological conditions. We hope to be able to follow this work along these lines in the near future.

<sup>1</sup> ABDERHALDEN: *Zeitschrift für physiologische Chemie*, 1905, xliv, p. 22; and 1902-1903, xxxvii, p. 484.

## OSMOTIC PRESSURE AND HEART ACTIVITY.

By A. J. CARLSON.

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THE experiments reported in this paper were made on the heart of *Limulus* and on the auricles of the tortoise. The experiments on the *Limulus* heart were undertaken primarily with the view of determining whether such small change in the osmotic properties of the blood as might be produced by the addition of certain drugs is a factor to be taken into consideration in studying the specific action of drugs on the heart. That the osmotic pressure change due to the solution of a drug in the blood is a negligible factor in the action of the drug on the heart appears to be the tacit assumption of pharmacologists. In fact, I am not aware that any specific observations have been made on effects of osmotic changes on rhythmically active tissues, such as the heart and the respiratory centre in the vertebrates. A second point to be determined on the *Limulus* heart was whether the nervous and the muscular tissues exhibit the same reactions or a different kind of reaction to osmotic pressure changes in the surrounding media. I have shown that in this animal the nervous and the muscular tissues may be so separated that the influence of a solution on the one tissue can be studied apart from that on the other. This paper reports the results only so far as regards the nature of the changes in the heart rhythm produced by osmotic changes of the surrounding medium, leaving for a later communication the question whether this is a factor in the action of any of the drugs on the heart.

The experiments on the *Limulus* heart brought out the fact that *hypertonicity of the medium depresses the rhythm primarily, while hypotonicity acts as a primary stimulus to the rhythm. This is also the effects of hypertonicity and hypotonicity of the medium in the case of the tortoise auricles. In Limulus these effects of hypertonicity and hypotonicity are true both for the heart ganglion and the heart muscle.*

The methods of preparing and suspending the *Limulus* heart so that the action of a solution on the heart muscle may be studied apart from its action on the heart ganglion have been described in my paper on the heat standstill of the heart.<sup>1</sup> The reader is referred to that paper for an account of these methods. The tortoise auricles were excised and suspended in a glass cylinder for graphic registration in much the same way that a segment of the *Limulus* heart was suspended, the cylinder being provided with an outlet and an inlet tube at the bottom so that the fluid surrounding the auricle could be changed at will.

In the experiments on the *Limulus* heart two methods of varying the osmotic pressure of the medium were employed, both giving the same results. The plasma or sea water was concentrated by boiling and reoxygenated by shaking with air; or the osmotic pressure was raised by the addition of sugar (cane sugar, *lactose*, *laevulose*) to the plasma or sea water. In the case of the *Limulus* heart sea water can be used as well as plasma or blood, because sea water appears to be entirely neutral during the first hours of its action, that is, neutral in the sense that the blood or the plasma is neutral. The activity of the heart is not altered on the heart being transferred from plasma to sea water or from sea water to plasma unless the bath in sea water is long continued.

In the experiments on the tortoise auricles Ringer's solution or  $\frac{9}{8}$  NaCl solution was used. The hypertonicity was obtained by the addition of cane sugar and the hypotonicity by dilution with distilled water.

#### THE INFLUENCE OF HYPERTONICITY ON THE HEART RHYTHM.

The influence of hypertonicity on the heart ganglion of *Limulus*.— When the plasma or sea water surrounding the ganglion is replaced by concentrated and reoxygenated plasma or sea water, the intensity of the nervous discharges from the ganglion is diminished almost immediately and remains thus weakened as long as the hypertonic solution is allowed to act on the ganglion. This appears to be the only change produced in the ganglion in case the hypertonicity is slight. This depression of the ganglionic rhythm is obtained with sea water concentrated to  $\frac{9}{10}$  of its volume, as well as by the addition of  $\frac{m}{10}$  cane sugar to the sea water or plasma. In the case of this rela-

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 207.

tively slight change in the osmotic pressure the rate of the ganglionic rhythm is not altered. The degree of depression of the strength of the nervous discharges from the ganglion is on the whole directly proportional to the degree of hypertonicity; the greater the concentration of the sea water or plasma, the greater the depression. This is true for the *Limulus* heart ganglion for concentrations up to twice that of the plasma or sea water. Greater concentrations were not tested for the reason that on further concentration of the sea water some of the salts begin to crystallize out especially at the optimum temperature of the *Limulus* heart ( $10^{\circ}$ – $15^{\circ}$  C.), and a molecular or  $\frac{9}{10}$  molecular solution of cane sugar in sea water is very nearly a saturated solution.

When the solution surrounding the ganglion is of a concentration of one and one-half up to double that of the normal, the rapidity of the ganglionic rhythm is diminished simultaneously with the depression of the strength of the rhythm. The reverse was never obtained, *i. e.*, a slowing of the rate without any alteration in the intensity.

The depressant action of the hypertonic solution on the ganglion appears within a few seconds after the ganglion is placed in the solution, and reaches its maximum usually within a minute or two, although in some cases it may take as long as five to ten minutes. This maximum depression is not long maintained. A very gradual recovery usually sets in, even though the hypertonic solution is not removed, but the depression is not fully removed except on replacing the hypertonic solution with normal blood or sea water. When the hypertonicity is slight, the degree of recovery is relatively greater than in the case of the more concentrated solutions. No experiment was extended over a longer period than one hour, so I am unable to say what course the ganglionic rhythm would follow beyond that period when subjected to the continuous action of a hypertonic solution.

While the degree of depression of the ganglionic rhythm is roughly proportional to the concentration up to twice that of the normal, *it is also dependent on the condition of the ganglion.* Thus a solution of one and one-half concentration produces greater depression on one ganglion than on another. And the relation is this: The weaker the ganglionic rhythm, that is, the "poorer" the condition of the ganglion, the greater the degree of depression by a given degree of hypertonicity. This is of interest in connection with the possible mechanisms by which a hypertonic media depresses the ganglionic rhythm, but it



FIGURE 1. — About seven-eighths the original size. Tracing from the anterior end of Limulus heart. The nerve cord isolated from the heart muscle posteriorly. The nerve cord surrounded by sea water. *X*, the sea water replaced by sea water concentrated one-third. *X'*, the concentrated sea water replaced by normal sea water. Showing depression of the ganglionic rhythm by the hypertonic solution

offers but slight aid in determining this mechanism or mechanisms, as we do not know what is the basis of the "poor" condition.

The recovery of the ganglionic rhythm on replacing the hypertonic solution by an isotonic one (plasma or sea water) is sometimes very rapid, at other times more gradual. The recovery may go to the extent of an improved rhythm for a time, that is, a stronger and a more rapid rhythm than that prior to subjecting the ganglion to the hypertonic solution.

The points to be noted in these experiments on the Limulus heart ganglion are: (1) hypertonic solutions depress the rhythm; (2) the depression is on the whole directly proportional to the concentration; (3) the degree of depression depends further on the "vital" condition of the ganglion; (4) the weaker concentrations depress only the strength of the rhythm, while stronger concentrations depress both the rate and the intensity; (5) the rhythm exhibits a partial recovery even during the time of action of the hypertonic solution.

These several points are illustrated by the typical tracings reproduced in Figs. 1, 2, and 3. These tracings are from the first two segments of the heart, the nerve cord or ganglion being isolated posteriorly and kept in a separate cylinder. In the tracing in Fig. 1 we have a typical depression of strength without any alteration of the rate of the ganglionic rhythm by placing the ganglion in sea water concentrated to one-third its bulk. The tracing in Fig. 2 *A* shows a similar depression — and gradual recovery of the rhythm — by sea water to which were added 0.6 molecular cane sugar; while tracing 2 *B* illustrates the depression of both rate and intensity of the rhythm by a hypertonicity twice the normal (molecular solution of cane



sugar in sea water). Tracing 3 *B* is from a heart in "poor" condition, 3 *A* from a heart in good condition. The ganglia of both

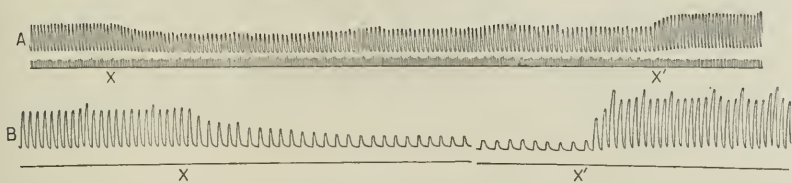


FIGURE 2.—One-half the original size. Tracings from the anterior end of the *Limulus* heart. The nerve cord isolated from the heart muscle posteriorly. Tracing *A*: *X*, ganglion surrounded by sea water + 0.6 molecular cane sugar. *X'*, normal sea water restored on the ganglion. Showing depression and gradual recovery of the ganglionic rhythm by the hypertonic solution. Tracing *B*: *X*, the sea water replaced by sea water saturated (molecular) with cane sugar. *X'*, the hypertonic solution replaced by normal sea water. Showing depression both of rate and intensity of the ganglionic rhythm.

hearts are placed in sea water of twice the normal concentration, the depressant action being greatest on the "poor" ganglion.

**The influence of hypertonicity on the heart muscle of *Limulus*.**—In speaking of the heart muscle it should be understood that I refer both to the muscle itself and to the motor nerves and nerve endings

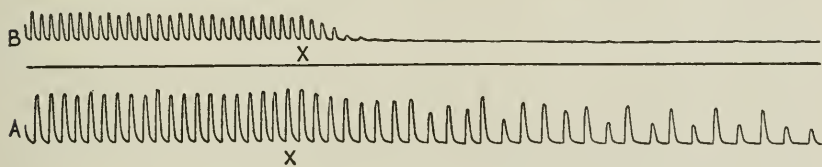


FIGURE 3.—Tracings from the anterior end of the *Limulus* heart. The nerve cord isolated posteriorly and kept surrounded with sea water. *X*, sea water replaced by sea water + molecular cane sugar. *A*, tracing from a heart in good condition. *B*, tracing from a heart in poor condition. Showing dependence of the degree of depressant action of hypertonicity on the condition of the ganglion.

in the muscle. We have at present no means of separating the one from the other. The effects of hypertonicity on the heart muscle is the same as on the heart ganglion, with this difference, that the muscle is usually less sensitive to the osmotic changes. When the plasma or sea water is replaced by concentrated plasma or sea water, or plasma to which sugar has been added, the amplitude of the beats is invariably diminished, and the depression is the greater the more concentrated the solution. The rhythm cannot be entirely suppressed by solutions of double the isotonic concentrations for at least one hour.



When the osmotic changes are confined to the muscle alone, the rate of the beats can, of course, not be altered, as the rate depends on the ganglion. No alteration of the rate was ever observed on bathing the heart muscle in the hypertonic solutions. Such change in the rate could under these conditions be brought about only by a reflex affecting the ganglion. Simultaneous tracings from the two ends of the heart according to the method described in my paper on the

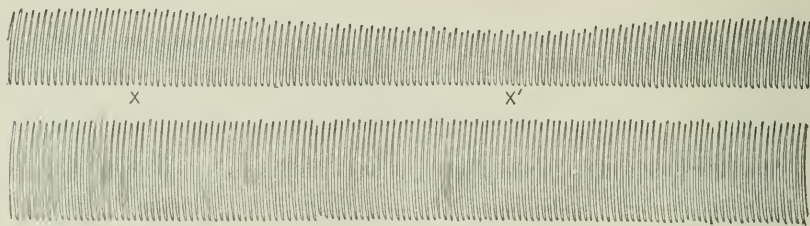


FIGURE 4.—One-half the original size. Simultaneous tracings from anterior (upper tracing) and posterior (lower tracing) end of *Limulus* heart. The nerve cord extirpated in the first two segments, leaving the two ends of the heart connected only by the lateral nerves. *X*, the sea water bathing the anterior end replaced by sea water + molecular cane sugar. *X'*, the hypertonic solution replaced by sea water. Showing depressant action of hypertonicity on the heart muscle (and nerve endings). The rhythm of the posterior end is not affected by these changes of the anterior end.

heart standstill were taken on several preparations on subjecting the anterior purely muscular (and nerve) end of the heart to the action of the hypertonic solutions. A tracing from one of these experiments is reproduced in Fig. 4. The lower tracing is from the posterior end, the upper tracing from the anterior end of the heart. It will be seen that the rhythm of the two ends is perfectly synchronous. The posterior end continues its rhythm absolutely unaffected while the beats of the anterior end are being diminished by the hypertonic solution, and restored to their original strength by the isotonic solution (sea water). The ganglion on the posterior end is therefore not affected by subjecting the anterior end of the heart to these changes. The depression of the beats of the anterior end is due to local action of the hypertonic solution on that end. The depressant action may be on the nerve endings, thus diminishing the strength of the nervous impulses reaching the muscle; or it may be on the muscle itself. There is no way of deciding between these two possibilities, for we know of no drug or solution or any other device that will paralyze the motor nerve endings in the heart muscle without injury to the heart muscle. It is possible that some light might be thrown on this

question by testing the contractility and excitability of curarized skeletal muscle when perfused with hypertonic solutions. Provisionally I have ascribed the depressant action of the hypertonic solutions on the ganglion free anterior end of the heart as due to the action of hypertonicity on the heart muscle itself. If this shall prove to be correct, we have the significant fact that the activity of the ganglion cells, a process presumably not involving changes of form, is affected in the same way as the contractility of the muscle, a process involving the change of form. This ought to be of some help in determining the mechanism by which hypertonicity depresses protoplasmic activity.

The fact that in *Limulus* heart the hypertonicity acts in the same way on the muscle (and nerve endings) as on the ganglion prevents the use of this heart in a way to explain the point of action of hypertonicity on the vertebrate heart, in which the nervous and the muscular tissues cannot be separated for experimentation.

**The influence of hypertonicity on the tortoise auricle.**—The experiments on the tortoise heart were undertaken mainly for the purpose of determining whether hypertonicity acts in the same way on the vertebrate heart as on the heart of *Limulus*. The auricle of the tortoise was chosen in preference to the ventricle, because the auricular walls are much thinner than the walls of the ventricle. Hence when the auricle is bathed in hypertonic or hypotonic solutions uniform osmotic conditions for all the muscle cells are much more readily established by diffusion than in the case of the ventricle. Some experiments were also made on the ventricle left in connection with the auricles, but prepared and fixed for graphic registration in such a manner that the solutions could be applied to the ventricle without affecting the auricles.

The hypertonicity was obtained by the addition of cane sugar to the Ringer's solution or the  $\frac{2}{3}$  NaCl solution. The Ringer's solution is the more favorable for the auricular rhythm, the strength of the beats diminishing much more rapidly in the pure sodium chloride solution. Contracture and tonus rhythms are also much more frequent in auricles suspended in the latter solution; but so far as the specific effects of hypertonicity are concerned the same amounts of cane sugar added to the Ringer's solution produced the same depression as when added to the pure sodium chloride. Two series of concentrations were tested, one twice the normal, the other one and one-half the isotonic concentration. Intermediate concentrations were not worked with.

Ringer's solution or  $\frac{n}{8}$  NaCl, to which  $\frac{m}{8}$  cane sugar has been added, depress the amplitude of the auricular beats, usually without altering the rate of the rhythm. If the rate of the rhythm is altered it is invariably in the direction of retardation. If the concentration of the medium surrounding the auricle is twice the isotonic ( $\frac{m}{4}$  cane sugar added), the rate of the beats is nearly always diminished at the same time that the amplitude of the beats is reduced. If the hypertonic solution is allowed to act on the auricle for ten to thirty minutes, a gradual recovery of the rhythm, particularly the amplitude of the beats, takes place. Complete recovery of the rate and strength of the rhythm takes place on replacing the hypertonic solution with Ringer's solution or  $\frac{n}{8}$  NaCl. The depressant action of hypertonicity is greater on some auricles than on others, just as was found to be the case in the *Limulus* heart.

There is thus a complete identity of the effects of hypertonicity on the *Limulus* heart and the tortoise auricle. A difference appears in case the hypertonic solution is allowed to act on the heart muscle alone (in *Limulus*), for in that case the rate of the beats cannot be altered; but when the whole heart of *Limulus* is involved, or the ganglion alone, the similarity between the influence of hypertonicity on the tortoise auricle and the *Limulus* heart is so close that the tracings in many instances cannot be told apart.

A tracing illustrating the influence of hypertonicity on the fundamental rhythm of the tortoise auricle is reproduced in Fig 5. At X the  $\frac{n}{8}$  NaCl is replaced by  $n$  NaCl +  $\frac{m}{4}$  C<sub>12</sub> H<sub>22</sub> O<sub>11</sub>. The amplitude of the beats diminishes before the rate is retarded; in fact, the retardation of the rate is very slight. The break in the tracing represents an interval of ten minutes. It will be seen that the amplitude of the beats has increased slightly. At X', the hypertonic solution is replaced by  $\frac{n}{8}$  NaCl, and the beats increase in strength till the normal amplitude is attained, the rate of rhythm being unaltered.

The hypertonic solution depresses not only the fundamental rhythm, but also the tonus rhythm, in case the latter is in evidence. This is true whether the tonus rhythm is or is not accompanied by the fundamental contractions. It is well known that tonus rhythms are of frequent occurrence in the tortoise auricles under various experimental conditions. In this series of experiments I have had three different auricles exhibit longer or shorter periods of tonus rhythms in the absence of the fundamental contractions. A hypertonicity of one and one-half or twice the normal gradually abolishes this tonus

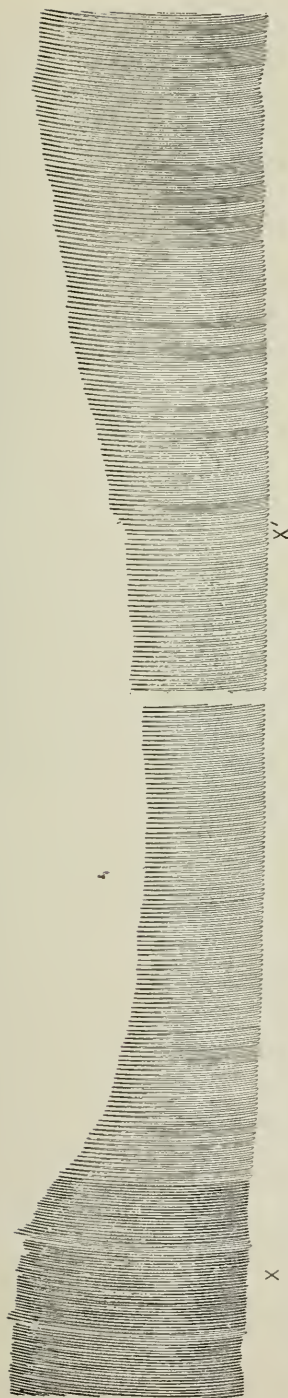


FIGURE 5.—One-third the original size. Tracing from the isolated and suspended auricle of the tortoise. Auricle beating in  $\frac{1}{8}$  NaCl at the beginning of the experiment.  $X'$ , the  $\frac{1}{8}$  NaCl replaced by  $\frac{1}{8}$  NaCl +  $\frac{m}{4}$  cane sugar.  $X'$ , the hypertonic solution replaced by  $\frac{1}{8}$  NaCl. Showing depressant action (mainly in the strength of the beats) of the hypertonic solution. The cut in the record represents an interval of ten minutes.



FIGURE 6.—One-half the original size. Tracing from the isolated and suspended auricle of the tortoise. Auricle in strong tonus and tonus rhythm while bathed in  $\frac{1}{8}$  NaCl at the beginning of the experiment.  $X'$ , the  $\frac{1}{8}$  NaCl replaced by  $\frac{1}{8}$  NaCl +  $\frac{m}{4}$  cane sugar.  $X'$ , the hypertonic solution replaced by  $\frac{1}{8}$  NaCl. Showing the depressant action of hypertonicity on tonus.



rhythm, and replacing the hypertonic solution by the isotonic solution usually brings the tonus contractions back. A tracing showing this influence of hypertonicity on the tonus rhythm is reproduced in Fig 6. The auricle from which this tracing was obtained was strongly contracted in addition to the tonus rhythm, so that the fundamental contractions are diminutive.

#### THE INFLUENCE OF HYPOTONICITY ON THE HEART RHYTHM.

**The influence of hypotonicity on the heart ganglion of *Limulus*.** — The effect of hypotonicity is uniformly the opposite of that of hypertonicity. Every degree of dilution from isotonicity down to distilled water acts as a stimulus to the heart ganglion of *Limulus*. When the ganglion is bathed in plasma or sea water diluted  $\frac{1}{8}$  to  $\frac{1}{5}$ , the strength of the nervous discharges is augmented without any attendant change in the rate of the rhythm. When greater dilutions are used, the rate is invariably augmented. If the rate of the ganglionic rhythm is greatly augmented, as in the case when the ganglion is placed in distilled water, the nervous discharges may appear to be diminished in intensity rather than increased, because the beats of the reacting anterior end usually become weaker. That may, however, be due to the fact that the muscle has less time to recuperate between each beat; the beats may thus be weakened, although the intensity of the nervous impulses causing the beats is actually increased.

In sea water or plasma of one-half the normal concentration the ganglion continues in activity for hours. There is a gradual "adaptation" of the ganglion to the new osmotic conditions, just as in the case of the hypertonic solutions, the stimulating action of the diluted plasma or sea water reaching its maximum in a few minutes. This maximum is soon followed by a return towards the normal rhythm. On replacing the diluted plasma or sea water with slightly more concentrated plasma or sea water or with normal plasma or sea water, the rhythm of the ganglion is depressed very much in the same way that it is depressed by being placed in an hypertonic solution.

When placed in distilled water, the ganglion is brought to a standstill in two to five minutes. A vigorous ganglion maintains its rhythm in distilled water for a longer time than a ganglion in "poor" condition. By "poor" condition I mean a ganglion exhibiting a relatively slow and feeble rhythm. This suggests that one factor in

such a "poor" condition of a ganglion is increased permeability of the cells, allowing water and crystalloids in solution to enter or leave the cells more quickly. A ganglion brought to standstill in distilled water becomes active again on being placed in plasma or sea water, provided the distilled water bath is not kept up for a longer time than ten to fifteen minutes. After a water paralysis the returning rhythm is always extremely rapid, but the strength of the nervous discharges as indicated by the amplitude of the muscular contractions is minimal. The intrinsic heart nerves are less sensitive to the action of distilled water than is the ganglion.<sup>1</sup>

The points to be noted in the influence of hypotonicity on the *Limulus* heart ganglion are: 1. Plasma or sea water of less than isotonic concentration augments the rhythm; 2. the augmentation is on the whole directly proportional to the dilution; 3. The dilution of one-half or more of the normal concentration augments both the rate and the intensity of the nervous discharges, while a less dilution usually augments only the strength of the discharges; 4. The recovery of the ganglion after paralysis in distilled water is marked by a very rapid rhythm, the strength of the nervous discharges being at the same time subnormal.

A typical tracing showing the stimulating action of hypotonicity on the *Limulus* heart ganglion is reproduced in Fig. 7 *A*. At *X* the ganglion is placed in plasma diluted one-third. The strength of the beats is almost immediately increased, while the rate remains unaffected.

**The influence of hypotonicity on the heart muscle of *Limulus*.** — When the osmotic changes in the direction of hypotonicity are con-

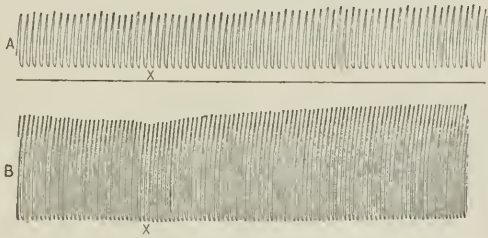


FIGURE 7.—One-half the original size. *A*, tracing from the anterior end of the *Limulus* heart. The nerve-cord isolated posteriorly. *X*, the normal sea water surrounding the ganglion replaced by sea water diluted one-third. Showing the stimulating action of hypotonicity on the ganglionic rhythm. *B*, tracing from the isolated and suspended auricle of the tortoise, auricle surrounded by  $\frac{2}{3}$  NaCl at the beginning of the experiment. *X*, the  $\frac{2}{3}$  NaCl replaced by  $\frac{1}{10}$  NaCl. Showing the stimulating action of hypotonicity, mainly on the strength of the rhythm.

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 118.



fined to the heart muscle and the nerve endings, the same stimulating action is observed as in the case of the ganglion, with the exception that the rate of the beats is never augmented. The reason for that is, of course, that the rate depends on the ganglion alone, while the strength of the beats may be altered either by altering the intensity of the nervous discharges from the ganglion or by altering the excitability and contractility of the heart muscle. It is well known that muscle absorbs water and ultimately loses its excitability and contractility when bathed in hypotonic solutions or in distilled water. The heart muscle of *Limulus* exhibits no exception to this rule. But before this depressant action of the hypotonic solutions sets in and water rigor appears, the strength of the beats is augmented. If the dilution is as great as five parts of distilled water to one part plasma, or if pure distilled water is used, the depressant action may appear without any primary stimulation. The distilled water may stop the muscular contractions before complete water rigor has set in. Whether this is due to paralysis of the nerve endings or to loss of excitability of the muscle itself cannot be decided at present. I have shown that it is not due to loss of conductivity of the nerve plexus.<sup>1</sup> In the case of dilutions down to one-half the isotonic there is no marked depression following the initial stimulation, although the amplitude of the beats gradually diminishes after the maximum height is attained; but on return to plasma or sea water of isotonic concentration, the amplitude of the beats is further diminished. The heart muscle is, on the whole, less sensitive than the heart ganglion to hypotonicity, but the same relation of degree of dilution to stimulating power observed in the case of the ganglion is also true for the muscle, within limits, that is, the greater the dilution the greater the augmentation of the amplitude of the contraction, except for dilutions approaching pure distilled water.

**The influence of hypotonicity on the tortoise auricle.** — So far as the experiments have been carried to date, hypotonicity acts the same way on the tortoise auricle as on the *Limulus* heart. *Diluting the Ringer's solution or the  $\frac{2}{3}$  NaCl augments both the fundamental rhythm and the tonus rhythm.* Solutions down to one-half the isotonic concentration usually augment the amplitude of the beats without altering the rate. Greater dilutions usually augment the rate in addition to increasing the amplitude. Dilutions greater than two parts of distilled water to one part of Ringer's solution have not

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 118.

been tried. The primary augmentation by the diluted solutions is quickly counteracted by replacing them with isotonic solutions. Within the range of the dilutions employed the stimulating power is, on the whole, directly proportional to the dilution, just as is the case in the *Limulus* heart. Hypotonic solutions tend to produce tonus rhythms in the tortoise auricles that do not exhibit any tonus variations in Ringer's solution or in  $\frac{2}{8}$  NaCl. And in case the tonus rhythms are present, diluting the medium augments both the rate and the amplitude.

A typical tracing showing the stimulating action of hypotonicity on the tortoise auricle is reproduced in Fig. 7 B. At X the  $\frac{2}{8}$  NaCl is replaced by  $\frac{2}{16}$  NaCl, with the usual results, the amplitude of the beats being augmented without attendant changes in the rate of the rhythm.

#### THEORETICAL CONSIDERATIONS.

It is well known that the osmotic pressure, that is, the molecular and ionic concentration, of the blood of most marine invertebrates and of many selachians is the same as that of the sea water, and that it varies directly with the concentration of the sea water. Garrey has shown that *Limulus* lives for many hours under such changed osmotic conditions that its blood is either diluted more than half or concentrated considerably above the normal.<sup>1</sup> Greene found that the blood of the Chinook salmon suffers a permanent dilution represented by a loss of 17.6 per cent of the salt contents of the blood when the fish has entered the rivers to spawn.<sup>2</sup> Yet the fish lives many weeks under these conditions. The concentration of the mammalian blood is more constant than in the case of the animals just referred to, but variations in the osmotic pressure of mammalian blood do occur. Wilson has recently shown that the electrical conductivity of human blood is subject to considerable variation, and probably a constant variation from the normal in certain diseases.<sup>3</sup> The electrical conductivity of the blood is, of course, not an infallible measure of the osmotic pressure, as the variations in the percentage of ions may be counterbalanced by an opposite variation in the percentage of sugar present. But it is probable that the conductivity changes represent parallel changes in the osmotic pressure of the blood. The influence

<sup>1</sup> GARREY: Biological bulletin, 1905, viii, p. 257.

<sup>2</sup> GREENE: Bulletin of the Bureau of Fisheries, 1904, xxiv, pp. 429-456.

<sup>3</sup> WILSON: This journal, 1905, xiii, p. 139.

of osmotic changes of the blood on nerve centres and muscular tissues, particularly the heart, is, therefore, a factor in the physiology of many, if not all animals. It may be a factor in some diseases.

The mechanism by which hypertonicity depresses and hypotonicity stimulates is almost certainly not the mere increase or decrease of external pressure on the cell membranes, with consequent loss of or imbibition of water. The permeability of the cell walls may be altered, thus inaugurating changes, quantitative and qualitative, in the crystalloids of the cells. A variable factor in the degree of ionization is introduced by concentrating or diluting the blood, except when the concentration is effected by the addition of sugar, and in that case it is by no means certain that the sole action of the sugar molecule is that due to the osmotic pressure factor.

*A priori*, one would have expected, other things being equal, the nervous and the muscular tissues to work best under the normal osmotic conditions; and perhaps that is true in the long run, for although a slight hypotonicity acts as a stimulus the end result may nevertheless be detrimental. Yet the fact remains that for a time, at least, the ganglion cell and the muscle cell work better when they contain more than the normal amount of water.

The fact that the depressant and the stimulating actions of osmotic changes are the greatest at the beginning might suggest that the stimulating and the depressant influences are associated with the changes leading to the establishment of a new state of equilibrium between the cell and its surrounding rather than with these new states of equilibrium themselves. But although hypertonicity depresses and hypotonicity stimulates, we cannot infer that imbibition of water stimulates and loss of water depresses the tissue, for when the *Limulus* heart ganglion that has been brought to a standstill in distilled water is transferred to plasma or sea water, the returning rhythm is of more than normal rapidity. The stimulating and depressant actions cannot, therefore, be causally related to imbibition or the loss of water, for in this case the ganglion is being stimulated while the water presumably is leaving the cells till the normal concentration of the cell contents is established.

## ON THE RESPIRATION OF THE HEART.

(WITH SPECIAL REFERENCE TO THE HEART OF LIMULUS.)

BY H. H. NEWMAN.

[From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.]

THE experiments reported in this paper were undertaken with the view of determining the relative sensitiveness of the *Limulus* heart to the lack of oxygen and to the presence of carbon dioxide, and, in the second place, to decide whether the heart ganglion and the heart muscle exhibit the same degree or different degrees of sensitiveness toward these conditions. The *Limulus* heart permits of conclusive experiments touching the latter question, because of the unique relation between the nervous and muscular tissues in this heart.

### I. THE EFFECT OF LACK OF OXYGEN ON THE HEART RHYTHM.

**Preparations and apparatus.**—Excised hearts of *Limulus* were prepared in the following ways:

(a) The ganglion was dissected free from the muscle of the posterior seven segments of the heart, the detached portion of muscle removed, leaving the ganglion in communication with the two anterior segments of the heart by means of the median nerve cord.

(b) The muscular portion of the third and fourth segments was removed, leaving the median and lateral nerves intact.

(c) The median nerve cord was dissected free from the first four segments of the heart and laid back. The muscular portion of the third and fourth segments was removed, leaving the two anterior segments connected with the ganglion only by means of the lateral nerve cords.

Practically identical results were obtained from the use of these three preparations, and hence the first method only will be considered in the following descriptions.

. This preparation was placed in the apparatus as described by Dr. Carlson<sup>1</sup> and shown in Fig. 1, the muscle *M* placed in the vessel *A* and attached to the lever of a kymograph, the thread *D* passing through a hole in the cork *C*<sup>1</sup>. A notch in this cork permits the

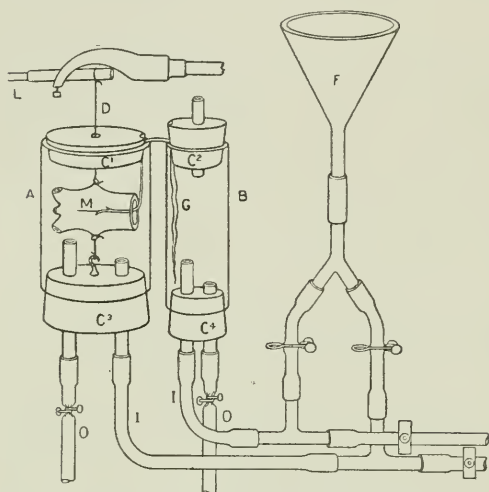


FIGURE 1. — Apparatus and preparation used in the majority of the experiments described in this paper. *A*, moist chamber containing the anterior two muscle segments (*M*) of the heart. *B*, moist chamber containing the ganglion *G* connected with *M* by means of the median nerve cord passing through notches in the corks *C*<sup>1</sup> and *C*<sup>2</sup>. *L*, lever of kymograph. *F*, funnel. *O*, tubes for withdrawal of liquid reagents. *I*, tubes for the supply of either gaseous or liquid reagents. *D*, thread connecting muscle to lever.

passage of the nerve cord into the vessel *B*, which is also provided with a notched cork *C*<sup>2</sup> containing a small exit tube. The ganglion *G* is allowed to lie against the side of the vessel *B*. Both vessels are provided with exhaust and supply tubes, *O* and *I*, which are arranged for supplying either gases or liquids.

The two vessels can be used as moist chambers by allowing a layer of sea water to remain in the bottom.

Sea water was used as a substitute for serum on account of its convenience. Garrey and Carlson have shown that sea water is practically isotonic with

*Limulus* blood, and is capable of maintaining the heart rhythm to the same extent as the latter, even stimulating slightly after periods of eight or ten hours' immersion.

The method of testing the activity of the heart in the absence of oxygen was to pass a continuous stream of purified hydrogen through the moist chambers *A* and *B*. This method has been used successfully in many similar experiments. The hydrogen was produced by the action of diluted sulphuric acid on zinc, and was purified by washing in sodium hydroxide, potassium permanganate, and distilled water.

<sup>1</sup> CARLSON: This journal, 1906, xv, p. 241.



The activity of the ganglion in the absence of oxygen and in an atmosphere of hydrogen.—A steady stream of hydrogen was passed through the chamber *B*, while the chamber *A* was filled with sea water.

The kymograph record (Fig. 2) shows that during nearly three and one-half hours the heart rhythm, instead of diminishing, steadily

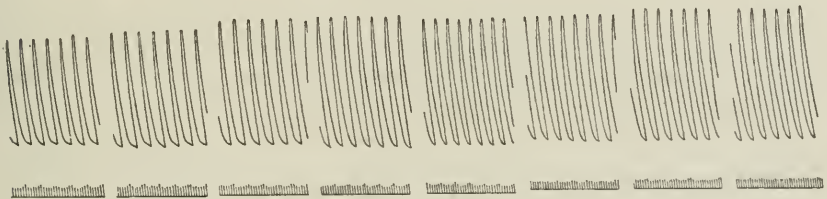


FIGURE 2.—Showing the effect of a stream of hydrogen on the ganglion during a period of three and one-half hours. Samples of the rhythm were taken at intervals of thirty minutes.

increased. The figure shows examples of the rhythm at intervals of thirty minutes. With the apparatus at hand it was not possible to maintain a perfectly steady and uninterrupted flow of hydrogen for longer periods. After thirty minutes the bubbles came at irregular intervals, and one could not be perfectly sure that no oxygen entered.

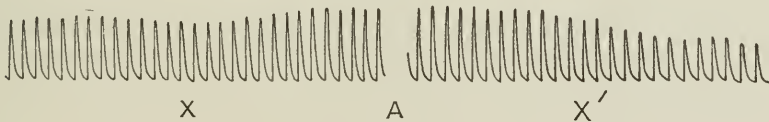


FIGURE 3.—Showing the effect of a stream of hydrogen acting upon the ganglion in a moist chamber during seven minutes. *X*, hydrogen turned on; *X'*, hydrogen turned off. The break at *A* represents a period of five minutes.

It will be noted that the frequency of the beats is practically uniform throughout the experiment, while the amplitude of the beats is markedly augmented.

This experiment was repeated on five other hearts, and the results were similar, except that, in the case of two weakly beating hearts, the hydrogen stream was unable to do more than maintain the initial rhythm. In these two cases the stimulating effect could be readily seen on shutting off and turning on the hydrogen stream. When the hydrogen was turned off the rhythm became quite suddenly weaker, and when it was turned on again the initial rhythm returned.

If the ganglion is exposed to moist air for an hour or more and a



stream of hydrogen then passed over it, the rhythm quickly increases in strength and maintains this strength as long as the hydrogen is present. When the latter is turned off, the rhythm quickly diminishes. Such a reaction is represented in Fig. 3.

As a variation of the first experiment the ganglion was immersed in oxygen-free sea water and the stream of hydrogen allowed to bubble through the liquid. In order to avoid a mechanical jar the supply tube was covered with gauze to break up the bubbles. The rhythm, as before, increased slightly in the course of about two hours, but when the hydrogen was turned off the heart quickly came to a complete diastolic standstill, recovering just as quickly when the hydrogen was turned on again. This was repeated several times with the same result. Other hearts failed to show such marked changes in rhythm, but the depression always followed the shutting off of hydrogen and augmentation the turning on again. In the first case it is probable that the ganglion was in an extremely sensitive condition. In all cases, however, the effect of hydrogen on the ganglion seemed to be a favorable one. It is difficult to account for this effect. On the whole I am inclined to believe that the influence is purely mechanical, and that the hydrogen washes away any volatile katabolic products of cellular activity that might be produced, probably carbon dioxide. The chemical inertness of the gas would preclude a chemical stimulus. It occurred to me later that the phenomenon might be due to the fact that the gas in the moist chamber was under pressure. Porter,<sup>1</sup> applying to the extirpated heart of the rabbit Haldane's experiment on the intact mouse, has shown that oxygen passed through the coronary veins under pressure is capable of maintaining the rhythm of the mammalian heart, and it might well be that pressure stimulates the rhythm in this case.

Sollmann's work on the mammalian heart<sup>2</sup> emphasizes the importance of pressure in the coronary vessels as a stimulus to the restoration of heart rhythm; but such an explanation could hardly apply to the experiments in hand, for the hydrogen is applied to the ganglion alone and no cardiac blood vessels are involved. He suggests, however, that the effect of the pressure as a stimulus to heart rhythm can be conceived more readily as originating in the nervous than in muscular structures. This phenomenon, then, might be due to a pressure on the nervous elements in the heart, and we are again led

<sup>1</sup> PORTER: This journal, 1898, i, pp. 516, 517.

<sup>2</sup> SOLLMANN: This journal, 1906, xv, p. 121.

to suspect that the stimulation produced by a rapid stream of hydrogen on the heart ganglion of *Limulus* might be due to gaseous pressure.

This theory was put to the test by using instead of hydrogen a rapid current of air. No stimulation was observable, and no changes in rhythm resulted from turning the stream on and off. The pressure idea, then, fails to explain the phenomena.

It is possible that hydrogen, on account of its highly diffusible character, may penetrate more readily than other gases into the substance of the ganglion and in this way serve to wash out the injurious products of cellular activity.

We may conclude from the preceding experiments that: (a) *The heart ganglion is comparatively indifferent to lack of oxygen, retaining its activity unimpaired when deprived of oxygen for several hours.*

(b) *Hydrogen exercises a favorable influence on the heart rhythm.*

**The activity of the heart muscle in the absence of oxygen and in an atmosphere of hydrogen.** — The ganglion in the vessel *B* was immersed in sea water, and the hydrogen stream was passed steadily through the moist chamber *A* containing the muscle. The rhythm was precisely similar to that produced by a stream of air, a gradual diminution in strength occurring during nearly three hours. This slight diminution in the amplitude of the beats always occurs unless the ganglion is artificially stimulated, and is doubtless merely the expression of a gradual muscular fatigue.

No changes in rhythm occurred when the stream of hydrogen was turned on and off or when an air stream was used as a substitute for hydrogen. So long as the conditions surrounding the ganglion remained constant, no alteration in the supply of air or hydrogen was able to affect the muscular rhythm.

It seems certain, then, that we have to reckon with the ganglion alone; and may eliminate from consideration the muscular elements of the heart. The whole heart may be used with the assurance that the effects noted may be attributed to the ganglion.

**The effect of lack of oxygen on the whole heart.** — In order to test the effect of lack of oxygen on the whole heart the following experiments were carried out. The hearts of six *Limuli* were excised. Three of these were suspended in a tightly sealed vessel containing 100 c.c. of oxygen-free sea water, prepared by boiling for an hour and rediluting to the original volume with boiled distilled water. The other three hearts were placed in a similar vessel filled with

ordinary unboiled sea water. Both vessels were kept at a uniform temperature by letting them stand in running sea water.

The following table will show that the hearts in both vessels beat progressively more and more slowly, and finally, after about twelve

TABLE I.

Time.	Hearts in O-free sea water.			Hearts in sea water.		
	A.	B.	C.	D.	E.	F.
hrs. min. Start	22	20	22	19	21	24
0 15	14	13	13	19	20	22
0 40	11	10	11	17	18	19
1 00	$9\frac{3}{4}$	$8\frac{1}{4}$	9	15	15	15
1 30	$7\frac{1}{4}$	7	7	13	13	$13\frac{1}{2}$
2 30	$5\frac{1}{4}$	5	5	10	11	$11\frac{1}{4}$
3 30	$4\frac{1}{4}$	4	4	$8\frac{1}{2}$	8	8
4 30	3	3	3	$7\frac{1}{4}$	7	$5\frac{3}{4}$
5 30	$2\frac{3}{4}$	$2\frac{3}{4}$	$2\frac{1}{2}$	$6\frac{3}{4}$	6	$5\frac{1}{4}$
6 30	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{4}$	$5\frac{1}{4}$	5	5
7 30	$2\frac{1}{2}$	$2\frac{1}{2}$	2	3	4	4
8 30	1	$1\frac{1}{2}$	$1\frac{1}{4}$	$1\frac{1}{2}$	4	$4\frac{1}{2}$
10 00	1	1	$1\frac{1}{4}$	stopped	3	$4\frac{1}{4}$
12 00	$\frac{1}{2}$	stopped	1	....	$1\frac{1}{2}$	$2\frac{1}{4}$
12 30	stopped	....	stopped	....	stopped	$1\frac{1}{2}$
13 00	....	....	....	....	....	1
14 00	....	....	....	....	....	stopped

hours, ceased to beat. The number of beats per minute is given for each observation, in a separate column for each of the hearts; A, B, C, being those in oxygen-free sea water, and D, E, F, being those in ordinary sea water.

It was noticed that bubbles of gas collected on the surface of the hearts in ordinary sea water and not on those in oxygen-free sea water. This might be accounted for on the supposition that carbon

dioxide is given off by all six hearts, but that it was more quickly absorbed by the gas-free liquid than by that charged with air. This inability to get rid of accumulated carbon dioxide might account in some measure for the rapid decline in the frequency of beats after two or three hours of activity.

It was also noticed that there was a marked depression in the frequency of beats during the first few minutes in the case of the hearts in boiled sea water, while the depression in the case of the hearts in ordinary air-charged sea water was very slight during the first thirty minutes or more.

The hearts A, B, and C, however, continued to beat on the average a little longer than the hearts D, E, and F.

The figures tend to show that the presence of oxygen promotes the rhythm slightly, but that its absence has no marked inhibiting effect on the heart activity.

It is remarkable that excised hearts are able to beat automatically in a medium devoid of oxygen for an average of over twelve hours. Had the vessels containing the hearts been kept in the refrigerator, they would undoubtedly have continued to beat for a much longer time, for the optimum temperature for the *Limulus* heart is considerably lower than that of ordinary running sea water.

**Whole animals in the absence of oxygen.** — Three young *Limuli* were placed in a tightly sealed vessel containing 100 c.c. of oxygen-free sea water, and observed at frequent intervals. After forty-five hours all three were perfectly dormant, moving neither appendages nor gills when mechanically disturbed, — a sure sign of complete paralysis.

All three were then removed into fresh sea water. One revived slightly, but the other two remained dormant and could not be stimulated to any display of reflexes. The dorsal ridge of the carapace of these two specimens was shaved off so as to expose the hearts. In each case the latter was seen to be beating rhythmically at a moderately slow rate, — 7 or 9 beats a minute. This experiment serves to show that in *Limulus*, as in the case of vertebrates, the heart is the last organ to be overcome by asphyxia, and that its ganglion — the seat of its automaticity — is strikingly more resistant to lack of oxygen than is the central nervous system.

## II. THE EFFECT OF CARBON DIOXIDE ON THE HEART RHYTHM.

All authorities agree that carbon dioxide has a deleterious effect on the heart rhythm, as the following extracts show:

Waller and Sowton<sup>1</sup> showed that the spontaneous beat of the normal excised heart is gradually inhibited by carbon dioxide. The beat is restored by displacing the carbon dioxide with air. The decline under the influence of carbon dioxide and the recovery on its displacement by air closely resemble the well-known muscarin decline and atropin recovery.

After complete inhibition of the spontaneous beat the muscle continues to respond to electrical stimuli, but if the action of carbon dioxide is continued for some time longer, the electrical response gradually diminishes and finally ceases entirely.

Magnus<sup>2</sup> (mammalian heart) shows that hearts that have beat rhythmically under the influence of perfusion with oxygen and hydrogen are promptly stopped when carbon dioxide is used.

McGuire<sup>3</sup> (frog's heart) has shown that blood rich in carbon dioxide paralyzes the heart, and he believes that the staircase phenomenon is due to the cumulative effect of gradually increasing amounts of carbon dioxide that are produced by muscular activity and cannot be eliminated from the tissues of the heart. The staircase phenomenon is, he believes, essentially a toxic phenomenon.

Saltet<sup>4</sup> (frog's heart), as appears in a later reference, agrees with McGuire.

Klug<sup>5</sup> (frog's heart) says that an accumulation of carbon dioxide paralyzes the rhythmicity of the heart. Before paralysis, however, the frequency of beats dropped from 44 to 22 per minute within a period of one hundred seconds.

Ringer<sup>6</sup> (frog's heart) states that salt solutions in which limited amounts of carbon dioxide are dissolved are prejudicial to the heart rhythm, when perfused through the heart.

Divine<sup>7</sup> (tortoise heart) shows that carbon dioxide paralyzes the

<sup>1</sup> WALLER and SOWTON: *Journal of physiology*, 1896, xx, p. xvi.

<sup>2</sup> MAGNUS: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlvii, p. 200.

<sup>3</sup> MCGUIRE: *Zeitschrift für Biologie*, 1901, xlii, p. 289.

<sup>4</sup> SALTET: *Ibid.*, 1905, xlvii, p. 312.

<sup>5</sup> KLUG: *Archiv für Physiologie*, 1879, p. 435.

<sup>6</sup> RINGER: *Journal of physiology*, 1893, xiv, p. 125.

<sup>7</sup> DIVINE: *Zeitschrift für Biologie*, 1905, xlvii, p. 335.



heart and produces the typical staircase phenomenon. The reverse staircase effect is seen on recovery after the removal of carbon dioxide.

Straub<sup>1</sup> (heart of *Aplysia*, an opisthobranch mollusc) — the only worker who has published results on the effect of carbon dioxide on the invertebrate heart — states that in general the effect of carbon dioxide consists of a lessening of the frequency of the beats accompanied by a definite increase in tonus. If the poison intensity of the carbon dioxide is high, the heart after a few beats comes to a standstill in complete systole. If, however, carbon dioxide is applied in small amount and for a limited time, the result is a gradual diminution in the amplitude of the beats accompanied by a marked lessening in their frequency and a slight rise in tonus. The rise in tonus seems to be regulated by the intensity of the carbon dioxide. This increase in tonus differs so markedly from conditions in *Limulus* that attention is directed to it here. The recovery is very slow, requiring nearly an hour during which the beats are very irregular with a tendency toward grouping.

The author found the heart of *Aplysia* very much more sensitive to small amounts of carbon dioxide than was that of the frog with which he had also worked.

The apparatus and preparation shown in Fig. 1 were used in these experiments. Carbon dioxide was applied in two ways, — as a stream of gas from a generator containing calcium carbonate and hydrochloric acid, and in the form of sea water charged from capsules of condensed carbon dioxide. The results from the use of the stream of gas were identical with those from the charged sea water. Consequently the latter form of carbon dioxide was used more frequently on account of its greater convenience.

**The effect of carbon dioxide on the ganglion.** — Carbon dioxide was applied to the ganglion in the vessel *B*, while the muscle was left in a medium of ordinary sea water. The result, as seen in Fig. 4, was an immediate primary stimulation resulting in an increased amplitude of beat, accompanied by a marked diminution in frequency. The primary stimulation lasts for only four or five beats, and then gives way to the typical inverted staircase phenomenon, followed quickly by a complete diastolic standstill. The records also show a gradual but marked lowering of tonus even after the standstill is reached.

<sup>1</sup> STRAUB: *Archiv für die gesammte Physiologie*, 1901, lxxxvi, p. 519.



After about two minutes sea water was used to wash out the carbon dioxide. Nearly three minutes elapsed before the rhythm commenced to return. The recovery is very gradual and somewhat irregular, but furnishes a good case of the staircase phenomenon, accompanied by a gradual raise in tonus. An invariable feature of the recovery is that, after the beats have regained their normal strength, or nearly so, they weaken again very perceptibly, — usually much more mark-

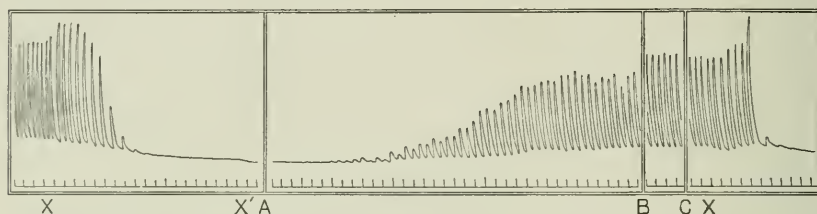


FIGURE 4. — Showing the effect of carbon dioxide on the ganglion. The gas was introduced at *X*, and allowed to remain for over two minutes, and then washed out with sea water at *X'*. The double line *A* after *X'* represents an interval of two minutes. The double line *B* and *C* represent intervals of two and one-half and two minutes respectively. On the right is seen the effect of a second application of carbon dioxide to the ganglion shortly after complete recovery. Time markings represent intervals of five seconds.

edly than is shown in Fig. 4, — and then gradually increase to a greater amplitude than normal. A period of irregularity follows, alternate beats being much shorter than the others. Complete recovery comes about by the gradual strengthening of the weak alternate beats until all beats are of equal strength.

If after a few minutes carbon dioxide is again applied to the ganglion, the primary stimulation occurs as before, but the staircase phenomenon is abbreviated, the ganglionic paralysis coming quite suddenly. Such a case is shown on the right-hand side of Fig. 4. The recovery is apt to be slower and less regular in case carbon dioxide is applied again too soon after the first treatment.

The process of paralysis and recovery can be repeated at intervals of fifteen minutes for three or four hours before the heart shows any symptoms of injury. After that time, however, it becomes more and more difficult to effect a recovery. If allowed to rest in a cool place for an hour or so, it may again be put through the same series of treatments as before.

This experiment was repeated many times, and the results were strikingly similar. The curve given in Fig. 4 represents an average case.

In a few cases, however, it was noticed that, after the rhythm had apparently ceased, a few weak spasmodic contractions occurred at fairly regular intervals. Fig. 5 shows a case of this sort. A rather weak dose of carbon dioxide was applied to the ganglion before it had completely recovered from a previous carbon dioxide paralysis. This tendency toward spasmodic contractions preceding complete diastole reminds one strongly of conditions found by Langendorff in the case of acute asphyxia of the respiratory apparatus of warm-blooded animals. There occurs at first an increase in the strength of the rhythm, followed by a standstill of varying duration. Then the rhythm returns for a few beats and passes suddenly into a permanent standstill. This he calls a grouped rhythm, and points out that similar phenomena have been observed by himself and others on the hearts of cold-blooded animals.

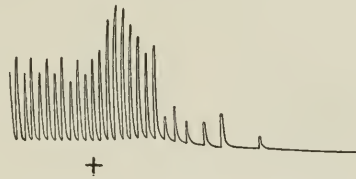


FIGURE 5. — Spasmodic contractions after standstill. At + a small amount of carbon dioxide was applied to the ganglion before complete recovery.

It appears, then, that carbon dioxide has a very quick and marked effect on the ganglion of the *Limulus* heart, producing, even when in weak doses, a complete paralysis. The muscle, which has not come in contact with carbon dioxide, is left inert and fully extended because it receives no impulses from the narcotized ganglion. That the effect is merely temporary and not especially injurious, is shown by the quick recovery that takes place on the removal of the narcotic carbon dioxide.

The primary stimulation observed when carbon dioxide is applied to the heart ganglion of *Limulus* is significant in view of the fact that this phenomenon has been repeatedly observed when animals are on the verge of asphyxia.

Both Langendorff and Winterstein testify that lack of oxygen produces stronger beats prior to a cessation of rhythm. These facts tend to show that the effect attributed to lack of oxygen is really due to an accumulation of carbon dioxide.

The phenomena that have been observed to characterize asphyxia are here seen to show a marked resemblance to those produced by the narcosis of the heart ganglion of *Limulus* by carbon dioxide.

Finally, it is important to note that the response of the ganglion to the presence of carbon dioxide is practically immediate. The effect is seen almost with the first bubble of gas that passes through the

sea water surrounding the ganglion. Hence the amount of carbon dioxide required to paralyze the ganglion is very slight.

**The effect of carbon dioxide on the cardiac muscle.**—While the ganglion was immersed in sea water in the vessel *B*, the muscle in vessel *A* was treated with sea water charged with carbon dioxide. No change in the rhythm was noticed for nearly a minute, — a period within which the ganglion would, under similar conditions, have been completely paralyzed. No primary stimulation was observed, but the

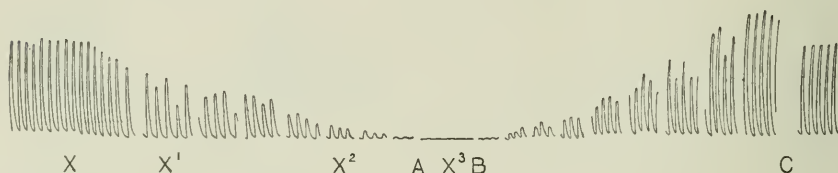


FIGURE 6. — Showing the effect of carbon dioxide on the anterior non-ganglionated portion of the heart, while the ganglion is immersed in sea water. Samples of the rhythm were taken at intervals of one minute except at *A* and *B*, which represent five-minute intervals, and *C*, which represents a fifteen-minute interval. Carbon dioxide was applied at *X* and repeated at *X*<sup>1</sup> and *X*<sup>2</sup>. The gas was thoroughly washed out with sea water at *X*<sup>3</sup>.

strength of the rhythm gradually and somewhat irregularly diminished, resulting in a complete diastolic standstill in about ten minutes after the application of the charged water. The latter was renewed several times during that period, as the rhythm showed signs of recovering its normal strength. Fig. 6 represents this process by means of samples of the rhythm every minute. After about one minute of standstill the carbon dioxide was washed out with fresh sea water, and the recovery began very slowly and irregularly, gradually increasing in amplitude until the beats were much stronger than normal. After about fifteen minutes of irregular rhythm the normal rhythm returned. If carbon dioxide is added a second time, the same process is repeated, but the recovery is apt to be delayed and the height of the rhythm to be more exaggerated. Usually a third treatment of the muscle with carbon dioxide results in a permanent paralysis from which the muscle will not recover.

It is important to note that only concentrated and frequently repeated doses of carbon dioxide produce paralysis in the muscle.

This seems to indicate that the amount of carbon dioxide produced by the cellular activity of the cardiac muscle would be entirely too small to have any paralyzing effect on the muscular element of the heart, while it may be sufficient to paralyze the ganglion.

**Effect of carbon dioxide on the whole heart.** — The whole heart was put in the vessel *A* and connected to the kymograph by the anterior segment as before. Carbon dioxide was applied, and the resulting curve was identical with that produced when the ganglion alone was treated. The ganglion became paralyzed before any effect could be produced on the muscle.

The ganglion becomes paralyzed in about one-tenth to one-fortieth the time required to paralyze the muscle; hence the muscle may be eliminated from consideration when the phenomenon of asphyxia is discussed, except when the condition of asphyxia is maintained for a considerable time.

**Effect of carbon dioxide on the whole animal.** — Three young Limuli were placed in sea water charged with carbon dioxide. They displayed violent activity for about a minute, then became gradually dormant. After a period of five to seven minutes they could no longer be stimulated to reflex activity. When put in running sea water, they revived in less than a minute. This process was repeated many times without in any way impairing the activity of the animals.

**Reflexes in the heart.** — To see whether the action of carbon dioxide on the muscle of the two anterior segments would induce any reflexes in the ganglion, the following preparation was fitted up in a modification of the apparatus shown in Fig. 1. Instead of the vessel *B* a wider and shallower vessel was used. The muscle of the third and fourth cardiac segments was removed, leaving the median and lateral nerve cords connecting the anterior and posterior regions of the heart.

The anterior portion of the heart was attached to one lever just as represented in Fig. 1, while the posterior region of the heart was attached at the seventh segment to a second lever. In this way the two separate records could be made, one directly above the other.

Carbon dioxide was applied to the anterior portion in vessel *A* with the result that the curve represented in Fig. 6 was duplicated, while beneath this the rhythm of the posterior end of the heart remained unaltered.

Even after a complete standstill of the anterior portion was effected there was no diminution in the rhythm of the posterior portion. This lack of reflex activity may have been due to the paralysis of the efferent nerve endings in the anterior portion or of the nerve cords connecting the two regions.

## III. THE CAUSES AND NATURE OF ASPHYXIA.

All workers agree that cardiac asphyxia is a paralysis brought about through the shutting off of the air supply from the respiratory apparatus, whether by strangulation, drowning, or the substitution of other gases for the respiratory media. That a lack of oxygen is indirectly concerned in producing the symptoms of asphyxia cannot be doubted, but there is much evidence to show that the direct cause of cardiac paralysis is not a lack of oxygen, for other rapidly changing gaseous media serve equally well to maintain the cardiac rhythm. On the other hand the blood of asphyxiated animals has been shown to produce the symptoms of asphyxia in living hearts, and this blood has been found to be richly charged with carbon dioxide.

The conclusions of some of the more prominent workers will serve to show the diversity of opinions held to-day.

Oehrwall<sup>1</sup> (frog's heart) is convinced that the small amount of carbon dioxide produced by the activity of the heart could not produce asphyxia such as he found when he deprived the heart of oxygen.

Klug<sup>2</sup> (frog's heart) suggests that the paralysis of the heart by carbon dioxide may be due to a stimulation of the inhibitory nerves.

Saltet<sup>3</sup> (frog's heart) comes to the conclusion that the pulsating heart produces in contact with its nutritive media carbon dioxide, which accumulates at first in the region where it is produced and then diffuses out to the layers of fluid that are in contact with the heart wall. If the nutritive medium is constantly changed, it will be able to carry away the products of activity as fast as they are produced, but, if it is unable to get rid of the carbon dioxide, saturation comes quickly. If the liquid medium is saturated with carbon dioxide, no more of the latter can be eliminated from the heart tissue, and accumulation takes place, producing paralysis. The rhythm returns if the carbon dioxide is removed either by diffusion or by massage. No recovery from paralysis can occur until the carbon dioxide is removed from the nutritive medium. Fatigue and exhaustion are identical processes. The muscle ceases to work only when its nourishing material is exhausted. The effect of

<sup>1</sup> OEHRWALL: *Skandinavisches Archiv für Physiologie*, 1898, viii.

<sup>2</sup> KLUG: *Archiv für Physiologie*, 1879, p. 435.

<sup>3</sup> SALTET: *Zeitschrift für Biologie*, 1905, xlvii, p. 312.



carbon dioxide is to alter the serum albuminate in such a way as to render it unfit for nourishing the heart tissues.

Langendorff<sup>1</sup> (mammalian heart) believes that a lack of oxygen produces the symptoms of asphyxiation, because it permits the accumulation of the products of activity that would otherwise be neutralized by oxidation. He also considers that asphyxiation is similar to narcosis and that death follows unless the narcotic influence is removed. The narcosis is produced by the overheating of the ganglionic elements of the heart, due to the accumulation of the products of activity that cannot be thrown off. The amount of heat necessary to paralyze the ganglion is insufficient to affect the muscle.<sup>2</sup> It is important to note that Langendorff attempts to locate the cause of asphyxiation in the paralysis of the ganglionic to the exclusion of the muscular elements.

These experiments on the heart of *Limulus* have shown that the ganglion is extremely sensitive to small amounts of carbon dioxide and the muscle very much less so. *Limulus* is a difficult animal to asphyxiate, and it is probable that the hearts of warm-blooded animals are very much more sensitive to carbon dioxide than the heart in *Limulus* or in cold-blooded vertebrates.

The phenomena of paralysis and spasmodic recovery seen in the *Limulus* heart when the ganglion is treated with carbon dioxide bear a striking resemblance to the phenomena exhibited by asphyxiated hearts of higher forms.

It seems very probable that *asphyxiation in Limulus consists of a paralysis of the cardiac ganglion, caused by an accumulation in the ganglionic tissue of carbon dioxide produced by cellular activity.*

#### SUMMARY.

1. The heart ganglion is comparatively indifferent to lack of oxygen, retaining its activity unimpaired when deprived of oxygen for several hours.
2. Hydrogen exercises a favorable influence on the heart rhythm.
3. The heart ganglion is very sensitive to carbon dioxide, showing a primary stimulation followed by a quick fall to diastolic standstill.

<sup>1</sup> LANGENDORFF: Archiv für Physiologie, 1893, p. 417.

<sup>2</sup> This view is contrary to the fact in the *Limulus* heart. This journal, 1906, xv, p. 215.



The recovery of the heart ganglion after carbon dioxide paralysis is rapid and may be repeated many times.

4. The heart muscle is very much less sensitive to carbon dioxide than is the ganglion, requiring strong and repeated doses in order to produce paralysis.

5. The heart ganglion of *Limulus* is much more resistant to lack of oxygen than is the central nervous system.

6. Asphyxia is believed to be due to an accumulation of carbon dioxide produced by the cellular activity of both muscular and ganglionic elements of the heart, but affecting chiefly the ganglionic element.

In conclusion I wish to express my thanks to Dr. A. J. Carlson for valuable suggestions and assistance during the progress of the work.

PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES  
OF MAGNESIUM SALTS.—II. THE TOXICITY OF  
INTRAVENOUS INJECTIONS; IN PARTICULAR THE  
EFFECTS UPON THE CENTRES OF THE MEDULLA  
OBLONGATA.

BY S. J. MELTZER AND JOHN AUER.

[From the Rockefeller Institute for Medical Research, New York.]

IT is very little known among medical men that magnesium sulphate, which is so widely and frequently used as a purgative, is actually a very poisonous substance. The physiological literature contains, however, a few records demonstrating the toxicity of these salts in intravenous injections. These observations were made incidentally in investigations upon saline purgatives. In these experiments the animals seemed to stand the intravenous injection of any amount of sodium sulphate without detrimental results, whereas the injections of magnesium sulphate proved to be invariably fatal to the few animals experimented upon. All the six dogs in which Leubuscher<sup>1</sup> injected magnesium sulphate, for the purpose of studying its purgative action in this mode of administration, died either during the injection or soon after. Leubuscher assumed that death is due to the precipitation of the globulins of the blood by the magnesium sulphate. Hay<sup>2</sup> met with similar results in a few experiments upon cats and on one dog. The observations of that author led him to the view that magnesium sulphate affects chiefly the respiratory function.

In our first communication<sup>3</sup> we have stated among other things that subcutaneous injections of magnesium salts can be fatal to the animal if given in certain large doses. The salts seemed to affect mainly the respiration, which gradually became more and more shal-

<sup>1</sup> LEUBUSCHER: Virchow's Archiv für pathologische Anatomie, 1886, civ, p. 104.

<sup>2</sup> HAY: Journal of anatomy and physiology, 1883, xvii, p. 512.

<sup>3</sup> MELTZER and AUER: This journal, 1905, xiv, p. 366.

low until it ceased entirely; this took place invariably before the heart stopped beating.

In the present communication we shall report a series of experiments with magnesium salts given by intravenous injection. We studied in particular the effects of these salts upon the activity of the centres located in the medulla oblongata (the respiratory, vasomotor, and deglutition centres) and upon the irritability of some of the nerves intimately connected with them. The results of these and of some other incidental observations will be briefly recorded.

The experiments were made exclusively on rabbits. In all cases tracheotomy was made (to be in readiness for artificial respiration), one carotid artery and one external jugular vein were provided with cannulas, and several nerves were isolated. The animals were anesthetized during the operation by ether, and in a few instances received also small doses of morphine or chloral. Blood pressure was written by a mercury manometer, the tubing connecting it with the carotid artery being filled with a mixture of sodium carbonate and bicarbonate. For the graphic presentation of the respiration, the respiratory variations of the pressure of the pleural cavity were utilized by means of Meltzer's<sup>1</sup> cannula; in most of the experiments the negative pressure was completely restored by the methods described by Meltzer.<sup>2</sup>

The experiments were made on forty-nine animals. Of the magnesium salts we have again tested the sulphate as well as the chloride. The sulphate of magnesium, however, was employed more extensively. The salts were tested in various concentrations: from 25 per cent solutions of magnesium sulphate (or 19 per cent magnesium chloride) to solutions of only a fraction of 1 per cent. In most of the experiments the injections were made into the external jugular vein from a Mariotte burette. In a few instances the injections were made by a syringe through the marginal ear vein.

We may state at the outset that the great toxicity of magnesium salts was evident in nearly every experiment. The first and most striking effect was its influence upon the respiration; a comparatively very small dose would suffice to completely arrest that function. However, the toxicity does not depend alone upon the quantity of

<sup>1</sup> MELTZER: *Zeitschrift für Instrumentenkunde*, December, 1894.

<sup>2</sup> MELTZER: This journal (*Proceedings American Physiological Society*), 1898, i, p. ix.

the salts; the speed with which the injection was made was quite an important factor in the final result. The following experiments illustrate these statements. In Experiment XIII an injection of 1 c.c. of a 25 per cent solution of magnesium sulphate was made in about twenty seconds (see Fig. 1). There was a complete arrest of the respiration long before the injection was finished, and we may assume that it was accomplished perhaps only by a dose of 0.1 gm. of the salt.

At any rate, 0.25 gm. of the salt injected within twenty seconds abolished the respiration so completely that artificial respiration had to be carried on for about half an hour before spontaneous respiration set in again. In Experiment XXVII, in which a solution of 1.7 per cent magnesium sulphate was employed, 30 c.c. (0.5 of the salt) was injected within twelve minutes practically without any effect upon the respiration. In the same experiment 83 c.c. of the above-mentioned solution (1.5 gm. magnesium sulphate) was injected within about one hour with no apparent harm to the animal. In one experiment 0.25 gm. of the salt injected within twenty seconds was nearly fatal to the animal, it being saved only by prolonged artificial respiration, while in the other experiment a dose of the salt, nearly six times as large but injected within one hour, proved to be without any perceptible effect upon the animal.

The intravenous injection of magnesium salts causes, under certain conditions, also a considerable fall in the blood pressure. Here the speed of the injection is even of more importance than in the effect of the salts upon respiration. Figs. 1 and 2 illustrate that point.

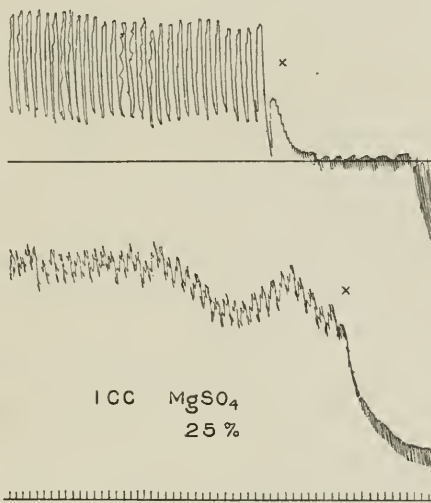


FIGURE 1.— Showing the effect of injection of 1 c.c. of 25 per cent magnesium sulphate solution into the jugular vein in about twenty seconds. Upper tracing presents respiration taken from pleural cavity; the up stroke, inspiration; the straight line, atmospheric pressure. Lower tracing presents blood-pressure curve. Crosses mark the identical points on both tracings. Time marking in seconds. Respiration was arrested immediately at the beginning of the injection.

In Fig. 1 (taken from Exp. XIII) at  $\times 1$  c.c. of 25 per cent of magnesium sulphate was injected into the jugular vein, as described above, in twenty seconds. Nearly simultaneously with the arrest of the respiration there was a drop of the blood pressure of about 40 millimetres. On the other hand, in Fig. 2 (taken from Exp. XL), in which the injection of 5 c.c. of a solution of 3.7 per cent magnesium chloride within about three minutes brought the respiration down nearly to zero, there was practically no lowering of the blood pressure; on the contrary, with the decrease of the respiratory amplitude, a moderate rise of blood pressure took place, owing apparently to the developing asphyxia, and with the improvement of the spontaneous breathing it disappeared again. The only noticeable effect in this experiment which the injection of the salt solution produced upon the blood pressure is the gradual disappearance of the respiratory and other variations of the pressure curve.

In a general way we may state that 0.1 to 0.2 gm. of the salts per kilo rabbit is capable of completely abolishing the respiration and profoundly affecting the blood pressure when administered intravenously by rapid injections. On the other hand, a rabbit will apparently stand even as much as 1 gm. per kilo if the intravenous injection be carried out sufficiently slowly.

The degree of the dilution in which the solutions of magnesium salts were employed had apparently no influence upon the extent of the toxic effects. We have to bear in mind, however, that, for mechanical reasons, with concentrated solutions no rate sufficiently slow can be attained which would make the injections harmless; and, on the other hand, for very dilute solutions it is difficult to attain a speed for the injections which would produce a definite toxic effect. In one experiment (XLVI) where we employed a solution of 0.3 per cent of magnesium sulphate, we could produce no effect upon respiration or circulation even with a considerable quantity and with the fastest flow from the burette.

Entering now upon a more detailed description of our results, we shall begin with some particulars of the effects of the injection upon respiration. When a moderately dilute solution (2 per cent or less) was injected with a relatively slow speed, there would be at first no visible effect; soon, however, the inspirations would become shorter and shorter until they gradually disappeared entirely. If the injection were discontinued shortly before or at least immediately on reaching the complete arrest of respiration, the inspirations would

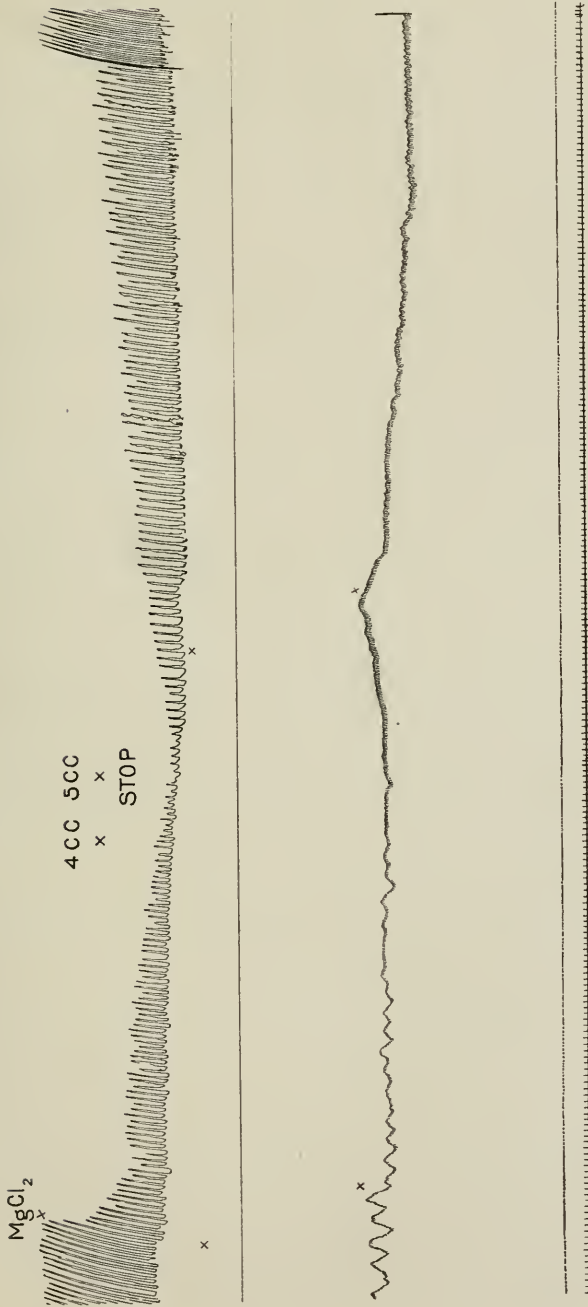


FIGURE 2.—One-half the original size. Showing the effect of slow injection of 5 c.c. of 3.7 per cent solution of magnesium chloride in about three minutes. Upper tracing presents respiration; inspirations, upward stroke (both vagi cut); straight line below, atmospheric pressure. Lower tracing, blood pressure; straight line below, base line. Time in two seconds. Crosses mark identical points in both tracings. Effect only on respiration; shortening of inspirations. No fall in blood pressure; slight rise due to shallowness of respiration.



soon begin again, first superficially, then gradually increasing until they reached their former size (see Fig. 2). The character of the descending line which connects the end points of the diminishing inspirations depends upon the character of the speed of the injections. If the injections are carried out with uniform speed, the line is nearly straight; otherwise the line is concave (increasing speed), or convex (decreasing speed), or irregular. The expirations generally are very little affected. However, when during the normal respiration the expiration was active, at the approach of the complete standstill there would be a shortening of the expirations.

Aside from the shortening of the respiratory amplitudes there would often be a tendency for the respiratory movements to become positive, especially when approaching their disappearance, that is, to get more or less below the atmospheric line. This occurred not only when the respiratory tracings were coming from the pleural cavity, but also when they were obtained from a large bottle which was connected with the trachea of the animal. This phenomenon might perhaps be explained by an overfilling of the viscera within the thoracic cavity with fluid, since it occurred mostly when large quantities of dilute solutions were injected in a rapid ratio.

In a few cases the effect of the injection upon respiration consisted also in moderately increasing the respiratory pause, especially when the effect was approaching the complete abolition of the respiration.

When the slow intravenous injections of the magnesium salts continued for a while, after all inspirations were obliterated, frequently at first a few struggling movements appeared, mostly of the character of brisk, active expirations, lasting altogether only a few seconds, to be followed immediately by absolute rest, the respiratory tracing presenting a straight line on the positive side of the atmospheric pressure. There would be no motor signs of asphyxia, no convulsion, no motion, no attempt to breathe, while at the same time the blood pressure might rise high up due to the effects of the asphyxiated blood. If the injection was soon stopped, a period of rest still followed, at the end of which slight respiratory motions would appear. The period of rest would be greater the longer the injection was continued after complete respiratory standstill was attained. The reappearing respiratory motions would at first frequently be separated by respiratory pauses of abnormal length. Gradually and slowly the inspirations would become deeper and more frequent, until finally the normal type of respiration would be reached again.

When concentrated solutions were rapidly injected, the inspiration would cease abruptly, to be followed immediately by absolute rest without a sign of a struggle of any kind. If a few c.c. of a 25 per cent solution of magnesium sulphate were rapidly injected, the animal would not regain its spontaneous respirations for quite a long time, even with continued artificial respiration; and if no artificial respiration were instituted, the animal would die without manifesting the slightest reaction.

To recapitulate briefly. Dilute solutions slowly injected would produce a gradual decrease of the inspirations, and, if continued, would lead up gradually to an inhibition of respiration, which after a very brief, slight struggle would terminate in complete rest. Concentrated solutions rapidly injected would terminate the respiration at once, and very frequently without the sequence of the struggles of asphyxia. *Under no circumstances did the injections of magnesium salts ever cause an increase of the inspirations in depth or frequency.* In other words: *Magnesium salts do not excite the respiratory function; on the contrary, if present in the blood in sufficient quantity they are capable of rapidly and completely inhibiting all respirations, and at the same time inhibiting all the excitation phenomena of asphyxia.*

We have also studied the relations of the pneumogastric nerve to respiration under the influence of the magnesium salts. It is well known that stimulation of the central cut end of the vagi affects the course and the character of the respirations. The nature of the influence which is brought about by stimulation has been for many years a subject of extensive controversy. We shall not enter here upon a discussion of that subject.<sup>1</sup> It will suffice to state that in the vast majority of our present experiments the effect of an electrical stimulation of the central end of one vagus (the other usually being uncut) in the normal animal was distinctly inspiratory in character. As a rule, with a moderate stimulus, the frequency of the respirations increases with a predominant shortening of the expirations, and with a stronger stimulus the respiration stood still for a brief period in a line approaching more or less the end points of inspirations. When now in our experiments a moderately dilute solution of a magnesium salt was slowly injected so as to bring the inspirations slowly to a minimum or even to an expiratory standstill, as described above, the inspiratory effect of a stimulation of the vagus would gradually turn more and more expiratory in character; *i. e.*, the line of stand-

<sup>1</sup> See MELTZER: *Archiv für Physiologie*, 1892, p. 340.

still would approach more and more the expiratory base. After the discontinuation of the injections and with the return of the inspirations to their normal extent, the effect of the stimulation of the vagi would also gradually resume again its inspiratory character (see Fig. 4). If, through a continuation of the injection of dilute solu-

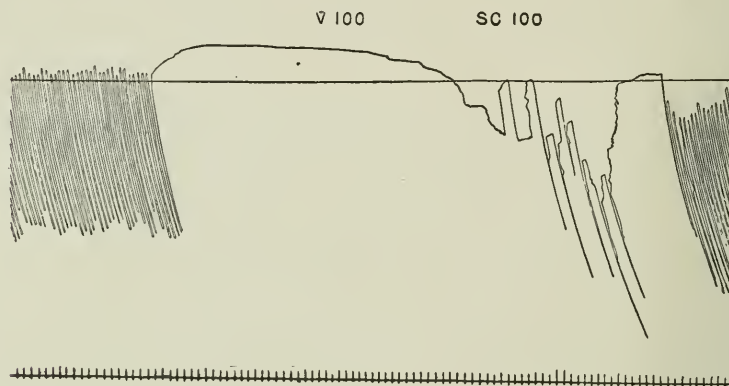


FIGURE 3.— Showing the effects of stimulation of the vagus and the sciatic nerves during respiratory standstill. It presents a section of a respiratory tracing after the animal had a rapid intravenous injection of 1.5 c.c. of 25 per cent solution of magnesium chloride. Artificial respiration was given (undulations below the atmospheric line). When interrupted, the respiration was marking nearly a straight line without any indication of a struggle; electrical stimulation of the central end of the vagus (*V*) with 100 mm. distance between coils had no effect, stimulation of the central end of the sciatic nerve (*SC*) with the same strength produced irregular active respirations.

tions or through a rapid injection of a concentrated solution, a prolonged standstill of the respiration was effected, no stimulation of the vagi with whatever strength would have the slightest effect upon the respiration, or, in other words, no stimulation of the vagi could bring out an inspiration ever so small during a standstill of respiration brought about by an injection of magnesium salts (see Figs. 3 and 7). However, as soon as spontaneous respiration sets in, after a somewhat prolonged standstill, the effect of the vagus stimulation would invariably be of an expiratory character, the respiration would stand still in expiration. Furthermore the effect of a vagus stimulation at this phase would often be to stop again the respiration for some time even after discontinuation of the stimulation (see Fig. 4), *b* and *c*.

We should say here that when we are speaking of an expiratory standstill or a standstill in expiration, we mean by it passive and not

active expiration; that is, we mean that state of a respiratory standstill which is due to neither inspiratory nor expiratory muscular contraction. The standstill is accomplished predominantly by an inhibition of the inspiratory muscular contractions.

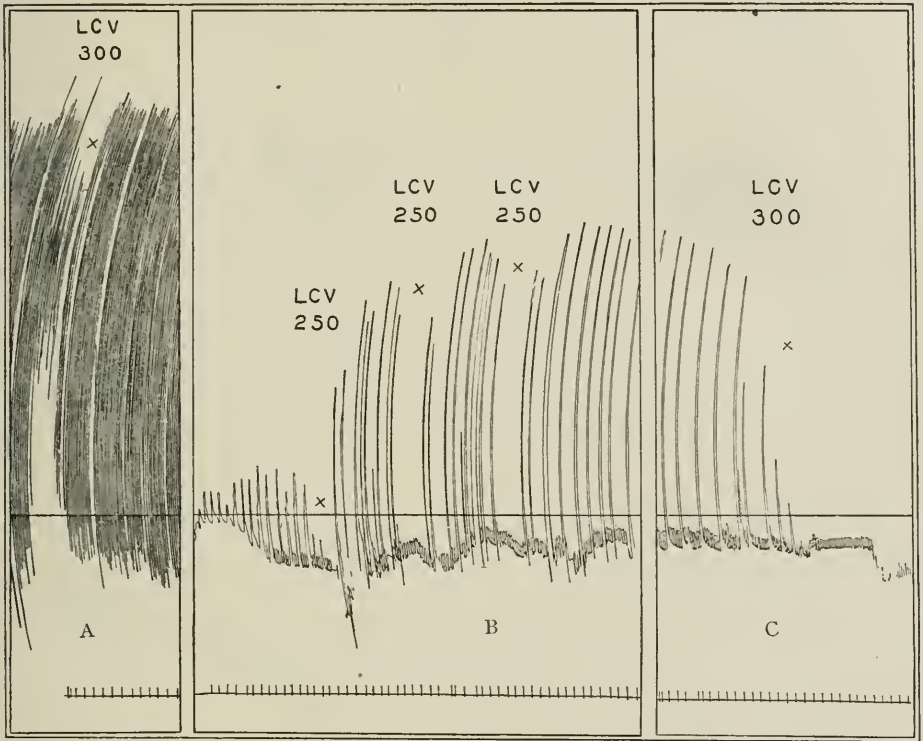


FIGURE 4. — The inhibitory effect of vagus stimulation at the resumption of spontaneous breathing after a prolonged standstill. *A* shows the inspiratory character of stimulation of central end of vagus (LCV) before the injection of the solution (1.7 per cent) of magnesium sulphate. Stimulation with 300 mm. distance between coils. *B*, at the resumption of spontaneous respirations (up stroke, inspiration). Stimulation with 250 mm. distance between coils causes distinctly expiratory standstill. *C*, very slow injection of the very dilute solution continued. Stimulation with 300 mm. distance between coils brought on continued expiratory standstill. Time, four seconds. Figures *B* and *C* also show the heart beats.

Our results with the stimulation of the vagi mean, then, that with the increase of the inhibitory effect of the magnesium salts upon respiration, the effect of stimulation of the vagi becomes more and more distinctly inhibitory in character, and continues in the same character for a short while after the resumption of spontaneous breathing.

Stimulation of the central end of a sciatic nerve was sometimes slightly but distinctly effective during a respiratory standstill. Fig. 3 shows such an effect; it presents a section of a tracing from such

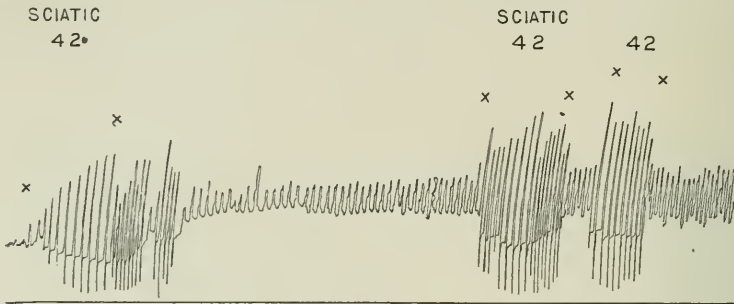


FIGURE 5. — Showing the effect of stimulation of the sciatic nerve in starting the respiration. After a prolonged complete respiratory standstill due to an intravenous injection of 11 c.c. of 5 per cent magnesium sulphate solution, a very strong stimulation (42 mm. distance of coils) of sciatic nerve started fairly regular respirations which continued in a superficial manner even after discontinuation of stimulation. A renewed stimulation intensified the respiration again.

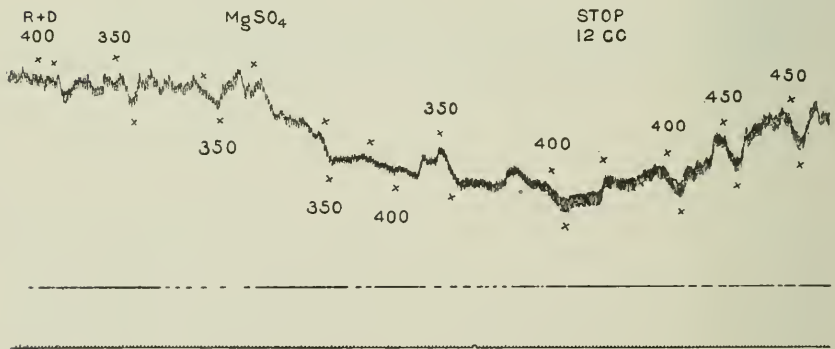


FIGURE 6. — Four-sevenths the original size. Showing behavior of depressor nerve after an injection of 12 c.c. of 5 per cent magnesium sulphate solution. Before injection stimulation with 400 mm. distance between coils had no effect, while soon after the injection even a stimulation with 450 mm. distance between coils brought out a distinct fall of blood pressure. Artificial respiration was given throughout the experiment. Time marked in two seconds.

a standstill after discontinuing the artificial respiration; the curve is nearly a straight line; stimulation of the vagus produced no effect, whereas stimulation of the sciatic produced a few active expirations. Sometimes stimulation of the sciatic started up spontaneous breath-



ing. Fig. 5 shows such an occurrence. The artificial respiration was discontinued, and the animal made no attempt to breathe sponta-



FIGURE 7.— Showing the effect of dyspnoic blood upon deglutition. The respiration was completely abolished for about half an hour. Upper tracing shows artificial respiration with Porter's small tambour; inspiration, down stroke. The present section shows a stoppage of the artificial respiration and stimulation of the central end of the vagus. Neither the asphyxia nor the stimulation had any effect upon respiration; the tracing shows a straight line. But the blood pressure rose, and on the height of it spontaneous swallowing occurred, which is marked by a brief fall of blood pressure. This tracing illustrates also two points made by one of us (M.) twenty-three years ago: that asphyxia causes "spontaneous" swallowing, and that with each act of deglutition there is a fall of blood pressure even in tracheotomized animals.

neously. The sciatic nerve was then stimulated with a strong current, which immediately brought out deep, regular respirations. After cessation of stimulation the spontaneous respirations continued, but in an abortive manner; a renewed stimulation of the sciatic improved the respirations again.

We may state here that *during a prolonged standstill of respiration the animal would as a rule be in a state of very deep general anesthesia. No voluntary or reflex motion of any kind was present.* A strong stimulation of the sciatic nerve, as stated above, would occasionally produce a moderate effect upon the respiration, and now and then would also affect slightly the blood pressure, but there would be not



the slightest manifestation of pain or any other reaction. The animal appeared as if deeply curarized, but stimulation of a motor nerve would bring out a motor response.

Regarding the vasomotor centre, we have already seen above that it is less readily affected by intravenous injection of magnesium salts than the respiratory centre. A dilute solution of the salt injected with moderate speed which is capable of bringing down the respiration to nearly a complete standstill might exert no important influence upon the blood pressure. A more concentrated solution injected with a rapidity sufficient to bring on a complete arrest of respiration without the struggles of asphyxia, will cause also a more or less distinct fall of blood pressure. The fall, however, is, as a rule, not very great, varying perhaps between 30 and 50 per cent of the original pressure, and is not long lasting; on the contrary, if artificial respiration is not soon instituted, it might rise above the original height, due, in the first place, to the developing asphyxia. Here we meet again with a striking difference between the vasomotor and the respiratory centres. The dose of magnesium salts which arrests the respiration and inhibits all the respiratory manifestations of asphyxia reduces the vasomotor tonus, but does not deprive the vasomotor centre of its sensitiveness to the action of the asphyxiated blood.

A rapid injection of the concentrated solution of magnesium salts causes sometimes a sudden fall of the blood pressure almost to the base line, and in this state even the asphyxiated blood seems to have very little effect upon the blood pressure. The presence of a large quantity of magnesium salts in the blood seems to abolish also the sensitiveness of the vasomotor centre to the stimulating effect of asphyxiated blood. The abolition, however, is rarely as complete as in the case of the respiratory centre, and, as a rule, is only of short duration. At any rate, the blood pressure under all circumstances begins to rise again long before there is any sign of a reawakening of the respiration.

In almost all cases the artificial respiration kept the blood pressure more or less down. The degree of that depression, however, depended considerably upon the degree of the original fall due to the injection of the magnesium salts. When the effect of the magnesium was not strong and the vasomotor mechanism was not deprived of its sensitiveness to asphyxiated blood, the depression due to the mechanical effect of the artificial respiration was not very great, and with the continuation of the artificial respiration it became gradually less

and less; the recovering vasomotor centre apparently asserted itself against the mechanical interference.

The appearance of a strong rise of blood pressure soon after the moderate fall was, in some cases at least, probably due to some other factor besides the stimulating effect of asphyxiated blood. We have stated above that the tubing connecting the carotid artery with the mercury manometer was filled with a mixture of carbonate and bicarbonate of soda. It may be assumed that in many cases of a noteworthy fall of blood pressure some of the sodium carbonate would thus get into the circulation, and we know already, since the days of L. Traube and through the recent investigations of Dawson,<sup>1</sup> that sodium carbonate causes a rise of blood pressure. The presence of this factor would explain the difference between the often very considerable rise of pressure after the first fall and the several moderate rises after each discontinuation of the artificial respiration. Under these circumstances it is very interesting to note that after the considerable falls of pressure, when surely a good quantity of sodium carbonate reached the circulation, the vasomotor centre does not respond with a rise of pressure. That means that in this instance the magnesium salts deprived the centre, at least temporarily, of its sensitiveness to asphyxiated blood as well as to the stimulating effect of sodium carbonate.

As is well known, stimulation of the central end of the vagus causes frequently a rise of blood pressure. In our experiments we have observed that a stimulation of the central cut end of the vagus caused in many instances a moderate but distinct rise of blood pressure even in cases in which the same stimulation had no effect upon the respiration, owing to a complete inhibition of the respiratory centre. The degree of the rise was usually smaller than the one observed before the injection. This observation illustrates two facts: 1st, that the effect of a stimulation does not depend upon the nerve trunk or nerve fibres, but upon the nerve centres in which these fibres terminate; 2d, that the rise of blood pressure upon stimulation of the central end of the vagus is due to a primary effect upon the vasomotor centre and is not a secondary effect due to respiratory changes.

We have stated above that strong stimulation of the sciatic nerve during a complete respiratory standstill had frequently a moderate but distinct effect upon the respiration. These stimulations sometimes produced a moderate rise of blood pressure. In one or two

<sup>1</sup> DAWSON: *Journal of experimental medicine*, 1905, vii, p. 1.

instances, however, in which the stimulations of the sciatic nerve produced active expirations, hardly any effect upon the blood pressure could be noticed.

Stimulation of the crural nerve which practically never had any effect upon respiration often produced a slight but distinct rise of blood pressure if the fall of pressure caused by the injection of the magnesium salts was not too great.

We have studied more extensively the efficiency of the depressor nerve after intravenous injections of magnesium salts. The results can be briefly stated as follows: In a large majority of experiments there seemed to be no diminution in the depressing effect of a stimulation of that nerve upon the blood pressure. Even during a complete and prolonged standstill of respiration and with a fall of blood pressure amounting to 50 per cent and more, a stimulation of the depressor brought out a further fall equal in value at least to that obtained before the injection of the magnesium solution with the same strength of stimulus. In the two or three instances in which a stimulation of the depressor nerve after the injection of magnesium salts did not produce any effect, the blood pressure was so low that even without the influence of magnesium stimulation of the depressor nerve could hardly reduce the pressure still more. And even in these cases, immediately after the moderate improvement of the blood pressure, a stimulation of the depressor promptly became effective. On the other hand, we have observed in a good many instances that, during an injection of magnesium salts, and especially immediately after it, a stimulation of the depressor nerve became perceptibly more effective; for instance, a stimulus of a strength which before the injection produced hardly any effect or no effect at all, produced immediately after the injection a perceptible drop in the blood pressure (see Fig. 6).

When we look upon the action of the depressor as an inhibition of the tonus of the vasomotor centre, we may express the last observation in the following terms: An intravenous injection of magnesium salts increases for a time the inhibitory influence of the depressor upon the vasomotor centre.

Our observations upon the vasomotor mechanism may be briefly recapitulated as follows: The vasomotor centre can be profoundly influenced by the intravenous injection of magnesium salts, but it is far less readily affected than the respiratory centre. *The effect is always in the nature of a depression and never excitation.* The respon-

siveness to the exciting effects of asphyxiated blood and of stimulations of sensory nerves is perceptibly reduced, whereas the responsiveness to the effects of stimulation of the depressor nerves is not only not reduced, but is rather perceptibly increased.

While studying the effects of intravenous injections of magnesium salts upon the vasomotor centre, we have of course made simultaneously some observations on the effect of the injections upon the heart. We have also studied the influence of these injections upon the effect of stimulation of the peripheral end of the vagus upon the rate and character of the heart beat. We intend, however, to deal with these subjects in a separate paper, and shall state here only casually that an injection of the magnesium salts, which was effective enough to produce a considerable fall of blood pressure, caused temporarily also a greater or less slowing of the heart beats, and that in none of the experiments in these series have we observed a reduction in the inhibitory effect of the cardiac vagus.

We also made some observations on the effect of intravenous injections of magnesium salts upon the efficiency of the centre of deglutition. Differing from the other centres of the medulla by not being in a state of permanent tonic activity, the efficiency of the centre of deglutition can be studied only by testing the reflexes which reliably induce it to its normal activity. It consists, as is well known, in the production of a series of consecutive, efficient contractions, beginning in the mouth and pharynx and ending with the cardia. Reliable methods of starting deglutitions reflexly are the mechanical stimulation of a certain area of the soft palate and the stimulation of the central end of the superior laryngeal nerve. The responsive spot on the soft palate is best reached by a probe introduced through an opening in the thyreo-hyoid membrane (Kronecker and Waschilieff). Deglutitions are sometimes produced also by a stimulation of the central end of the vagus nerve, and sometimes apparently "spontaneous" acts of deglutition appear in the first stages of asphyxia,<sup>1</sup> the dyspnoëic blood acting here as a stimulus, as it acts on the other centres of the medulla.

For the observation of deglutition the animal had to be out of the influence of ether, as anesthesia interferes greatly with the activity of the centre of deglutition.<sup>2</sup> Before each intravenous injection the reliable responsiveness to mechanical stimulus was ascertained, and the

<sup>1</sup> MELTZER: *Archiv für Physiologie*, 1883, p. 230.

<sup>2</sup> MELTZER: *This journal*, 1899, ii, p. 206.

strength of the electrical stimulus determined which promptly brought out a complete reflex deglutition. The results of these observations are briefly stated as follows. Magnesium salts in intravenous injections abolish reflex deglutitions. The deleterious effects are developed gradually. The contractions of each part become weaker before they disappear completely, and then the cardia, œsophagus, larynx, and pharynx stop participating in the contractions in the order named. The centre of deglutition is slightly more resistant to the effects of magnesium salts than the respiratory centre. When the injections are made with a moderately dilute solution and with a moderate speed, the centre of deglutition is still capable of responding promptly to reflex stimulation while the inspirations are already rapidly disappearing, and even after complete respiratory standstill sets in a few seconds might yet pass before the centre of deglutition becomes completely disabled. If the injected dose of the salt was large enough to produce a prolonged respiratory standstill even after the discontinuation of the injection, it would frequently occur that the centre of deglutition would recover its excitability before the reawakening of the respiratory centre. In general the centre of deglutition is more resistant than the centre of respiration; it succumbs later and recovers earlier. During such periods of comparative responsiveness of the centre of deglutition while the respiration is inactive, it would happen that the asphyxia caused a spontaneous act of deglutition (see Fig. 7). On the other hand, when the centre of deglutition was deprived of its excitability by injections of magnesium solutions, which were neither long nor strong enough to cause a prolonged respiratory standstill, it would occur that spontaneous respirations set in before the centre of deglutition recovered its normal activity. It seemed that the centre of deglutition when once put out of function, no matter by what dose, always required some time—eight to ten minutes—to recover again. The recovery of the function of deglutition occurs in the reverse order of that in which it disappears. First there would be only a faint elevation of the larynx, etc.; then a strong elevation of the larynx would be followed by a feeble contraction of the œsophagus, etc., until each act of deglutition would be completed by a normal contraction of the cardia.

The centre of deglutition, however, is distinctly less resistant to the effects of magnesium salts than the vasomotor centre. In many instances after injection of magnesium salts no deglutition could be elicited while the blood pressure still remained unaffected or was only slightly depressed.



The slow recovery of the function of deglutition in cases in which the respiratory centre resumed its activity again, brought out an observation which deserves to be recorded. As is well known, Rosenthal discovered that stimulation of the central end of the superior laryngeal nerve inhibits the inspiration; it causes an expiratory standstill. In graphic records the respiratory tracing produced by that stimulation does not present a straight line; it is interrupted by what may appear as shortened respiratory motions. They are due, as is now generally assumed, to the motions caused by the acts of deglutition which are simultaneously brought out by the stimulations of the superior laryngeal nerve. Some of these motions are the

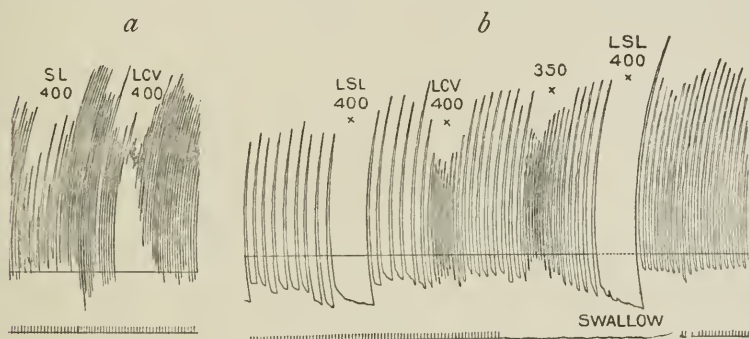


FIGURE 8.— One-half the original size. Showing the effect of stimulation of the superior laryngeal nerve. *a*, superior laryngeal nerve (*SL*) was stimulated with 400 mm. coil distance. Then 9 c.c. of a dilute solution (1.7 per cent) was injected, which inhibited the respiration and required artificial respiration for a short time. Soon after recovery of spontaneous breathing the superior laryngeal was stimulated again with the same strength. *b* presents that section of the tracing. Stimulation with 400 mm. coil distance produced swallowing, but with absolute inhibition of any respiration, — no “Schluckathmung” present.

so-called deglutition respiration (*Schluckathmung*). We need not enter here further into a detailed discussion of that subject. Now, in a number of instances in our experiments in which the electrical stimulation of the superior laryngeal nerve with a certain strength produced a tracing looking like anything but a straight line, the stimulation of that nerve with the same strength soon after resumption of spontaneous respiration would produce complete expiratory standstill, the tracing presenting a straight line indeed. This would happen especially when the stimulation would not yet produce any acts of deglutition, which would mean that nerve fibres within the larynx which affect the respiration had already recovered, while



those producing deglutition were still inhibited. However, that straight line occurred sometimes even when the stimulation did already produce deglutitions, though they made no mark on the tracing (see Fig. 8). We are therefore compelled to assume that at the beginning of the recovery of the respiration from the effect of the magnesium, the laryngeal nerves are enabled to inhibit the respiratory motions accompanying the deglutitions, just as it enables the vagus to display its inhibitory powers at the beginning of the recovery of spontaneous breathing.

Finally, we may be permitted to record the result of one experiment. In the face of the great toxicity of magnesium salts by intravenous (and subcutaneous) injections, the question naturally arose why these salts are not poisonous when given by mouth. When absorbed from the gastrointestinal canal, the magnesium salts have to pass through the portal vein and the liver before reaching the nervous system. It is generally assumed that many poisonous substances lose their toxicity on this path. Could it be true also of magnesium salts and hence the innocuousness when taken by the mouth? We have tested this not very probable assumption by injecting magnesium solutions through a mesenteric vein. In this experiment the toxicity of the salts was apparently as great as when injected through the jugular vein.

#### SUMMARY.

Magnesium salts in intravenous injections are very toxic, even in small doses. The first effect is upon the respiration, which becomes completely inhibited. The respiratory centre is deprived of its responsiveness to asphyxiated blood, and the reflex effect of sensory stimuli is greatly reduced. Magnesium salts favor the inhibitory effects of the respiratory fibres of the vagus. Prolonged artificial respiration restores earlier or later the respiratory function. Repetition of injections within a relatively short time increases the susceptibility of the animal to the toxic effect.

Large doses of the salt injected with rapid speed affect also the tonus of the vasomotor centre. That centre, however, is rarely deprived of its responsiveness to asphyxiated blood, the fall of the blood pressure is rarely extreme, and the toxic effects upon the vasomotor function disappear soon, at least a good deal sooner than the effect upon the respiratory function. During the toxic effect upon

the vasomotor mechanism, the vasoconstrictor effect of sensory stimuli is distinctly reduced, but not the effect of the stimulation of the depressor nerve. On the contrary, the vaso-inhibitory effect of that nerve is apparently rather increased.

Magnesium salts inhibit also the activity of the centre of deglutition. This centre is less resistant than the vasomotor centre, but is slightly more resistant than the centre of respiration. The activity of the several parts of the path of deglutition is not inhibited at once, but consecutively from below upward; the parts regain their activity also consecutively, but in reverse order. For some time after resumption of spontaneous respiration on stimulation of the superior laryngeal nerve the respiratory motions called "swallowing-respiration" (Schluckathmung) are inhibited even when stimulation causes swallowing.

During the complete inhibition of respiration by the intravenous injection of magnesium salts the animal is in a state of deep, general anesthesia, with complete relaxation of all muscles.

An animal might stand very slow injections of a very dilute solution of magnesium salts without harm. In our experiments 1 gm. of magnesium sulphate per kilo animal injected within one hour produced no perceptible harmful effects. This is perhaps important for the explanation of the apparent harmlessness of the administration of magnesium sulphate by mouth. The absorption from the gastrointestinal canal is perhaps so slow as not to introduce into the circulation more than the above-mentioned innocuous dose.

## THE ADAPTATION OF THE SALIVARY SECRETION TO DIET.

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IN recent years a number of papers have been reported which show adaptation by the pancreas to different diets. Wassilief<sup>1</sup> and Lintwarew<sup>2</sup> have shown that in dogs the quantity of pancreatic juice, as well as the quantity of enzymes, is dependent upon the food of the animal.

Ellinger and Cohn<sup>3</sup> have recently shown that the human pancreatic juice is affected by the diet, — that is, a proteid diet increases the proteolytic ferment; a fatty diet increases the lipolytic ferment; but a starchy diet, in his case, did not increase the amylolytic ferment, as Walter<sup>4</sup> had shown in the dog. Bainbridge<sup>5</sup> has shown that lactase, which is not normally found in the pancreatic juice of the dog, is produced when a dog is fed for two or more weeks on a milk diet.

The object of this paper is to show that diet has an effect on the amylolytic power of the saliva of the dog. It might be mentioned that even in the newest text-books it is stated that the saliva of the dog is inert or relatively inert. In all our experiments the saliva of the dogs was found to be active, but varying considerably in its amylolytic power, in different animals.

Chittenden, Mendel, and Jackson,<sup>6</sup> in their work on the influence of alcoholic drinks on digestion, point out that these substances have a stimulating effect on the quantity of saliva. There is also an

<sup>1</sup> WASSILIEF: *Archives des sciences biologiques*, St. Petersburg, 1893, ii, p. 219.

<sup>2</sup> LINTWAREW: *Biochemisches Centralblatt*, 1903, i, p. 201.

<sup>3</sup> ELLINGER and COHN: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 28.

<sup>4</sup> WALTER: *Ebenda*, 1899, vii, p. 1.

<sup>5</sup> BAINBRIDGE: *Journal of physiology*, 1905, xxxi, p. 98.

<sup>6</sup> CHITTENDEN, MENDEL, and JACKSON: *This journal*, 1898, i, p. 164.

increase in both the organic and inorganic constituents. Curiously enough, no mention is made of a change in its amylolytic power. Chittenden and Richards,<sup>1</sup> in their paper on the variations in the power and composition of the saliva, show that saliva is more powerful before activity of the gland than after activity, that alkalinity increases with the amylolytic power, and that the saliva has variations throughout the day.

#### METHODS.

Some dogs were fed on a bread diet, and others on a meat diet. "Baker's" stale bread was used. This was a little dry but otherwise good. It was broken up, and over it just a little, weak, warm meat broth was poured. The dogs were given plenty of water. The meat fed dogs were given both raw and cooked meat, which was sometimes ground and sometimes not. They were also given some meat broth. These animals did not thrive as well as the bread-fed dogs, but they kept a good appetite.

After feeding one dog for fourteen days on a meat diet, he was anesthetized, and the salivary glands on one side were taken out with aseptic precautions and the wound closed by catgut sutures. A small local infection followed, but this healed readily after the sutures were taken out and the wound opened. The removed glands were treated as described below, and the dog was then put on a bread diet for fourteen days. The experiments were carried out as follows:

- A. On street dogs just brought in (unknown diet).
- B. On bread-fed dogs.
- C. On meat-fed dogs.
- D. On the operated dog.
- E. On dogs on our diet, principally mixed diet.

In most cases both the saliva and the watery extracts of each of the salivary glands were used. A number of experiments were made with saliva only, on dogs which were kept on the diet given the dogs in our animal house. This diet consists principally of bread, with a small amount of meat broth and some ground meat. These dogs invariably had a saliva with strong amylolytic powers. The glands were weighed, ground in a mortar with washed sand, and then mixed with a definite quantity of distilled water, so that in each experiment

<sup>1</sup> CHITTENDEN and RICHARDS: This journal, 1898, i, p. 461.

each individual gland has the same percentage of gland tissue. This was filtered through cheese-cloth and added to a definite quantity of 1 per cent starch paste. This mixture was placed in Erlenmeyer flasks, and then put into an incubator registering 40° to 42° C. The saliva was used in definite amounts, diluted once with water and mixed with a known amount of 1 per cent starch paste.

In comparative experiments the proportions were always the same. The amylolytic power of the saliva and of the extracts was determined by both qualitative time tests and quantitative reduction tests, Haines' solution being used in each case. This is a modification of Pavy's test, and in our hands gives admirable results.

The qualitative time tests were made to show how soon the mixture showed a reduction. 1 c.c. of the solution was mixed with 5 c.c. of Haines' solution, boiled and then cooled under the tap. If the cuprous oxide came down at once or after standing a few hours, the test was considered positive and the time noted.

#### RESULTS.

The results of our experiments are shown in the following tables :

- A. Experiments on street dogs, unknown diet.** — Saliva was used which was collected from a cannula placed in the submaxillary duct by stimulation of chords. The extracts were made as described in "Methods," the same percentage of gland by weight to the amount of water being used, as is given in the following experiments :

*Dog I.* — All glands showed trace of sugar in 120 minutes ; submaxillary saliva showed trace of sugar in 90 minutes.

*Dog II.* — All glands showed trace of sugar in 240 minutes ; submaxillary saliva showed trace of sugar in 120 minutes.

*Dog III.* — All glands showed trace of sugar in 90 minutes ; submaxillary saliva showed trace of sugar in 60 minutes.

All gave good reductions later. No quantitative determinations were made. Also, in these dogs the saliva was collected which dripped from the mouth during the operation of putting the cannula in the submaxillary duct and the following stimulation. This is the mixed saliva from the sublingual and parotid glands. When treated like the submaxillary saliva, it showed approximately the same amylolytic power. For this reason, in the following experiments, the submaxillary saliva was taken as representing the mixed saliva of all the glands.

B. Experiments on Meat-Fed Dogs. — Dogs Fed on Meat for 17 Days.

Gland or saliva.	Wt.	Amt. of water.	Amt. of 1% starch paste.	Time, in minutes, of first reduction.	Amount of sugar.
Sublingual . . .	gm. 1.470	c.c. 33	c.c. 60	60. Trace of sugar on standing. 105. Heavy reduction at once. Much sugar.	gm. } 0.049
Submaxillary . . .	5.9	65	135	60. Trace of sugar on standing. 105. Heavy reduction at once.	} 0.054
Parotid . . . . .	1.6	31	62	50. Trace of sugar on standing. 90. Heavy reduction at once. Much sugar.	} 0.071
Submaxillary saliva	c.c. 5	5	20	30. Good reduction at once. Much sugar.	Gave no starch or dextrine. Reaction with iodine.

C. Experiment on Dog Fed on Meat for 21 Days.

Gland or saliva.	Wt.	Amt. of water.	Amt. of 1% starch paste.	Time, in minutes, of first reduction.	Amount of sugar.
Sublingual . . .	gm. 1.1	c.c. 25	c.c. 45	60. No sugar. 120. No sugar. 180. Trace of sugar on standing.	gm. } 0.0133
Submaxillary . . .	3.3	37	74	60. No sugar. 120. No sugar. 180. Trace of sugar.	} 0.0199
Parotid . . . . .	1.8	35	69	60. No sugar. 120. Sugar on standing.	} 0.021
Submaxillary saliva	c.c. 2	2	8	60. Trace of sugar only. 120. Good reduction. Much sugar.	Still showed blue with iodine.



**D. Experiments on Operated Dog. — Fed First on Meat for 14 Days.**

Gland or saliva.	Wt.	Amt. of water.	Amt. of 1% starch paste.	Time, in minutes, of first reduction.	Amount of sugar.
Sublingual . . .	gm. 0.450	c.o. 10.0	c.c. 18.0	60. No sugar. 120. No sugar. 180. No sugar. 240. Trace of sugar on standing. 60. No sugar. 120. No sugar. 180. No sugar. 240. Trace of sugar on standing.	gm.  No quantitative determination made.  0.029
Submaxillary . .	4.55	50.0	103.0		
Parotid . . . .	Mistake made in taking out gland				
AFTER OPERATION DOG WAS PLACED ON BREAD DIET FOR 14 DAYS.					
Sublingual . . .	gm. 0.43	c.c. 9.5	c.c. 17.1	60. No sugar. 120. Sugar at once. 60. No sugar. 120. Good reduction. 60. No sugar. 120. Trace of sugar.	0.041
Submaxillary . .	0.41	46.0	90.0		
Parotid . . . .	0.65	12.4	24.8		

**E. Experiments on dogs fed on our diet.** — The saliva was taken from the submaxillary duct, as described above.

The extracts were made in the same manner as described in "Methods."

The same percentage of gland by weight to the amount of water was used, as is given in Experiments **B**, **C**, and **D**.

The "time of the first reduction" was approximately the same as "the time of the first reduction" in the experiments on the bread-fed dogs.

The object of the experiment on these dogs was merely to show that on the diet which is given our animals, there is a strong amylolytic power in their saliva. This is only a further proof that the diet has an effect on the secretion of the salivary glands, as this diet consists chiefly of bread, with a minimum amount of meat and meat broth.

No quantitative determinations were made, as they would not have added anything to the results found in **B**, **C**, and **D**.

By consulting the above tables, it is seen that the salivary glands adapt themselves to the diet. Probably in all dogs there is an active ptyalin, but it is relatively inert as compared to human saliva. In Experiment **B**, sugar appears in the bread-fed dogs much sooner than

in the meat-fed dogs. This is also shown in Experiment C. It is further seen that the extract of the glands in the meat-fed, operated dog shows only a small amylolytic power, while extract from the glands after the same dog had been fed on bread shows a much greater amylolytic power. Both the saliva and gland extracts of dogs fed on our diet show a much greater amylolytic power than those of street dogs on an unknown diet.

The saliva is more active than the gland extract, and the latter responds to the diet like the glands themselves.

It is a well-known fact that extracts of glands in general are less active than the secretions. It may be that the gland contains a proferment. This may account for the latent period being longer in the gland extract than in the saliva itself, as the proferment must first be changed into the active ferment. It is conceivable that this adaptation occurs in the human being if a proper diet is given; namely, that on a starchy diet the amylolytic power of the saliva is increased. This is being investigated, and the result will be reported later.

## THE INVERSION OF STARCH BY PLATINUM BLACK.

BY C. HUGH NEILSON.

[From the Physiological Department of St. Louis University.]

THE use of catalytic agents in commercial chemistry has overthrown many of the older and more expensive methods. The action of catalytic agents has another interesting relation, as it has been shown that the actions of many enzymes can be simulated by certain catalytic agents, especially metals in a colloidal condition or in a finely divided form. Many experiments have been undertaken to prove the similarity between catalytic action and enzymatic action; but none need be mentioned in this paper except the work of Rayman and Sulc,<sup>1</sup> who have shown that finely divided metal, such as platinum, palladium, etc., can cause the inversion of cane-sugar. This change, which can also be produced by acids and also by the enzyme, invertase, is a hydrolytic cleavage of a disaccharide molecule into two monosaccharide molecules.

As the inversion of starch by the enzyme ptyalin is also a hydrolytic process, it occurred to me that the hydrolysis of starch might be brought about by finely divided platinum in the form of platinum black. In carrying out the experiments to prove this point, the following method was used:

The starch paste used in these experiments was made in the ordinary way. It was thoroughly boiled in order to sterilize it, and then boiled distilled water was added, to keep the concentration of the solution of a definite strength.

The flasks in which the mixture of starch paste and platinum was placed were sterilized by boiling and rinsed with sterile water. The platinum was weighed out in definite amounts and mixed with known quantities of starch-paste solution of a definite concentration. The flasks were stoppered with sterile stoppers, and placed in an incubator registering 40° C. A control flask was always made to determine if

<sup>1</sup> RAYMAN and SULC: *Zeitschrift für physikalische Chemie*, 1892, xxxi, p. 262.

any hydrolysis had taken place from any cause. The flasks were shaken at intervals in order to keep the platinum suspended in the solution as much as possible. It should be mentioned that different preparations of platinum have different degrees of activity in bringing about the hydrolysis of starch.

The rapidity and amount of hydrolysis were tested both qualitatively and quantitatively by the Haines method. The qualitative tests were made in order to determine how soon the mixture would show a reduction of Haines' solution. They were carried out by carefully opening the flask and taking out with a graduated pipette 1 c.c. of the solution. This was mixed with 5 c.c. of Haines' solution, boiled and cooled under the tap. A precipitate appearing at once on boiling or on cooling and standing until the precipitate of cuprous oxide had settled, was considered positive.

The quantitative determinations were made in order to determine the amount of hydrolysis of the starch in a given time. These determinations were made by the Haines method, which is a modification of Pavy's method. This method is just as accurate and is carried out more easily than Fehling's method. The reducing sugar which was produced is probably maltose, as will be proved farther along in the paper. It must be remembered that the reducing power of maltose on copper solutions is approximately one-third less than that of dextrose. This connection was made in these experiments.

A second method for determining the rapidity of hydrolysis of the starch was the use of the iodine test. This test was made at the same time as the qualitative tests for sugar. A solution of dilute, "wine-yellow" Lugol's solution was added drop by drop to 1 c.c. of the filtered starch solution, and the resulting color noted. It is necessary to use several drops, as achrodextrine, if present, will take up the iodine and no color will result, although starch and erythrodextrine may be present. More must be added, and if there be erythrodextrine present, a pinkish to a deep-red color will develop; and then, finally, if starch be present, the blue color of iodide of starch will appear. A rough, quantitative determination of the amount of hydrolysis of the starch may thus be made, if we note the number of drops used when the color appears.

*Experiments. Effect of time on the amount of hydrolysis.*

Dec. 12. — 8 A. M. 100 c.c. of 1 per cent starch paste was mixed with 8 gm. of platinum black and placed in the incubator.

Dec. 12. — 6 P. M. Trace of sugar found, as shown by the reduction test.

Dec. 13. — 8 A. M. Good reduction test. Test with iodine. 1 drop iodine, no color appeared; 2 drops iodine, a red color; 4 drops iodine, a blue color.

Dec. 15. — 8 A. M. A quantitative determination gave 0.027 gm. sugar. Test with iodine. 4 drops iodine, no color appeared; 5 drops iodine, a red color; 8 drops iodine, a deep red color. No blue could be obtained.

Dec. 16. — 8 A. M. A quantitative determination gave 0.039 gm. sugar. No color could be produced with iodine.

In these quantitative determinations the solution was taken out of the flask after thorough shaking to insure a homogeneous mixing of the platinum. The solution was then filtered, and the determination made with the filtrate. By this method of procedure the remaining part of the solution contained the same percentage of platinum and starch as at the time of the first determination. The second quantitative determination was made with this remaining solution.

*The effect of concentration of starch solution.*

Dec. 24. — 5 P. M. No. I. 100 c.c. of  $\frac{1}{2}$  per cent starch solution were mixed with 7 gm. of platinum black.

Dec. 24. — 5 P. M. No. II. 100 c.c. of 2 per cent starch solution were mixed with 7 gm. of platinum black.

Dec. 25. — 8 A. M. No. I. gave good reduction test for sugar; No. II. gave no reduction. Iodine test. No. I. 1 drop iodine, no color appeared; 2 drops iodine, a purple color; 3 drops iodine, a deep blue color. No. II. 1 drop iodine, a blue color.

Dec. 26. — 8 A. M. No. I. Quantitative determination gave 0.021 gm. sugar. Iodine test. 4 drops gave blue color. No. II. 98 c.c. of the solution partially decolorized 10 c.c. Haines' solution. But little sugar present.

Dec. 28. — 8 A. M. No. I. Quantitative determination gave 0.025 gm. sugar. No. II. Solution had already been used. Iodine test. No. I. 6 drops iodine, no color appeared; 7 drops iodine, purple color; 8 drops iodine, blue color. No. II. Solution had already been used.

*The effect of the amount of platinum black.*

Dec. 18. — 8 A. M. No. I. 50 c.c. of 1 per cent starch solution were mixed with 0.5 gm. platinum black.

Dec. 18. — 8 A. M. No. II. 50 c.c. of 1 per cent starch solution were mixed with 0.5 gm. platinum black.

Dec. 18. — 5 P. M. No. I. No reduction. No. II. Good reduction.

Dec. 19. — 8 A. M. No. I. No reduction. No. II. Good reduction.

Dec. 20. — 8 A. M. No. I. Trace of sugar shown by reduction. No. II. Heavy reduction.

The thought occurred to me that possibly the platinum black might contain bacteria which produced the hydrolysis. To avoid this, the platinum was sterilized by boiling in distilled water. It was then filtered and then sterilized in a hot-air chamber. Everything was prepared with aseptic precautions. To insure the solutions remaining sterile, a small crystal of thymol was placed in each flask. This substance has no retarding action on catalysis by platinum. In fact it might be said to be unnecessary to observe aseptic precautions, as the platinum itself, when it is kept thoroughly suspended in the solution, is bactericidal. With the above precautions hydrolysis occurred as before.

In all the above experiments control experiments were made, and at no time was there any sugar produced as shown by a qualitative test.

*The nature of the sugar produced.*

The polarimeter was first tried, but the solution being cloudy, due to the starch itself, and also to some of the platinum which passed through the filter, the polarimeter could not be used. Barfoed's reagent was next used. This, as is well known, is not reduced by pure lactose and maltose. The test was made by mixing 8 c.c. of Barfoed's reagent with 2 c.c. of the solution. The mixture was kept at a temperature of 100° for one hour. The different tests showed a very little reduction of the reagent, where the same amount of the solution showed a heavy reduction with Haines' solution. The small amount of reduction that took place may be due to the inversion of the starch or dextrine by the acetic acid in Barfoed's reagent.

The osazone by the phenyl-hydrazine test showed the characteristic melting-point of maltosazone. The inference, then, is that the sugar formed is maltose. Some dextrose may be present, which possibly might be produced by the inverting action of platinum on maltose.

It is thus seen by these experiments that platinum black can hydrolyze starch, and in all probability produces maltose. Furthermore, the reaction is evidently retarded by the products of the hydrolysis. For example, on December 26, .021 gm. sugar were produced; and on December 28, forty-eight hours later, the amount was but .025 gm.

It is further seen that the higher the concentration of the starch solution, the slower the action of the platinum. All these facts show the similarity between the action of platinum and the action of diastatic enzymes on the hydrolysis of starch.





## A NEW METHOD FOR INDICATING FOOD VALUES.

By IRVING FISHER.

[*Yale University.*]

UNTIL recent years any accurate record of diet, whether for purposes of statistics or prescription, has been impossible, owing to the lack of any adequate knowledge of the chemistry of foods. But to-day, by the aid of the elaborate tables of food values by Atwater and Benedict in America, and of mineral salts by Koenig in Germany, and also by the aid of coefficients of digestibility, it has become possible to keep a fairly accurate account of the constituents and calorific value of food. And yet two obstacles remain which have hitherto proved insuperable to the great majority of physicians. These are the tedious character of the calculations necessary for obtaining the required food values and the personal inconvenience to the subject necessitated by weighing his food. In the present paper a method is suggested by which both of these difficulties may be overcome.

The tables of Atwater and Benedict give the percentage by weight of proteid, fat, and carbohydrate in each kind of food. In order to use these tables the procedure which has been necessary hitherto is to weigh the food which is eaten, and to multiply the weight of each food by the three percentage figures just mentioned, and thus obtain the weight of proteid, fat, and carbohydrate; then to multiply the weight (in grams) of proteid and carbohydrate by the factor 4.1 in order to obtain the calorific value of these elements; and to multiply the weight of fat by the factor 9.3 in order to find the calorific value of fat. The results express the food value in "large calories" of proteid, fat, and carbohydrate, and the sum of these three is the total calorific value of the food. It is evident that when this procedure is carried out for all foods ingested, or food materials used in the kitchen, the labor involved becomes very arduous.

In order to simplify the problem, Dr. J. H. Kellogg of the Battle Creek Sanatorium devised a table in which, instead of the percentages

of Atwater and Benedict, the number of calories of proteid, fat, and carbohydrate for each food-stuff is specified. In addition to this, he provided his patients with a menu card on which the weight of each food as served at the table, and the calories of proteid, fat, and carbohydrate are printed. All the weighing is done in the kitchen, and the patient needs merely to check on his menu the articles eaten. His physician then makes the remaining computation, which consists in adding the calories of proteid, fat, and carbohydrate, and totals.

The method to be presented in the present paper is designed to still further save labor and at the same time to *visualize* the magnitude and proportions of the diet. It is similar to that just described in that it measures the food by calories instead of by weight, but differs from it in that it substitutes for the ounce, as the fundamental element, a "standard portion" of 100 calories. In order to carry out this method, foods should be served at the table in "standard portions" or simple multiples thereof. The amount of milk served, instead of being a whole number of ounces, should be 4.9 ounces, — the amount that contains 100 calories. This "standard portion" constitutes about two-thirds of an ordinary glass of milk. Of the 100 calories which it contains, 19 will be in the form of proteid, 52 in fat, and 29 in carbohydrate. In other words, of the food value of milk, 19 per cent is proteid, 52 per cent fat, and 29 per cent carbohydrate. The three methods which have been described may be contrasted as follows:

COMPOSITION OF MILK.

Method of	Proteid.	Fat.	Carbohydrate.	Total.
Weight per cent . . . .	3.3	4	5.0	12.3 in 100 oz.
Calories per oz. . . . .	3.8	11	5.9	20.7 in 1 oz.
Calories per cent. . . . .	19.0	52	29.0	100.0 in 4.9 oz.

One advantage of the last method is apparent at once. It enables us to make a true comparison between different foods as to the proportions of proteid, fat, and carbohydrate. The other methods are misleading in this regard. For instance, though it is well recognized that milk is a higher proteid food than pecan nuts, yet if we compare milk and pecans on the basis of the first method, the method of "weight per cent," we shall find that the pecans appear to be three times as high in proteid, milk containing 3.3 per cent and pecans 11 per cent. And if a comparison be made by the second method, that of "calories per ounce," we find the same misleading result; milk contains 3.8 ca-

lories per ounce and pecans 11.2. But if we compare them on the basis of "calories per cent," we find that, while milk contains 19 calories of proteid out of each 100 of total calories, pecans contain only 6. In other words, milk is now seen to contain three times as much proteid as pecans, instead of *vice versa*. The paradox that pecans are higher in proteid per ounce but lower in proteid per 100 calories is due, of course, to the fact that pecans are a much more concentrated food than milk, the concentration being due both to the absence of water and the abundance of fat, — the most concentrated of the three food elements. It requires 4.9 ounces of milk to yield 100 calories, but only .46 of an ounce of pecans to yield 100 calories. The comparisons which the third method gives, therefore, are comparisons between the food values of 4.9 ounces of milk and .46 ounces of pecans, not between those of 1 ounce of each.

The weight of any food yielding 100 calories is thus a measure of the degree of concentration. The most concentrated of all foods is probably olive oil, which contains 100 calories in almost one-third of an ounce (.38 oz.). Watermelon represents a food at the opposite extreme, of which over  $1\frac{1}{2}$  pounds are required to yield the 100 calories. In order that the "calories per cent" method may be easily put in practice, it is necessary to have a table giving the weight constituting a "standard portion" (*i. e.*, the amount yielding 100 calories) and the calories of proteid, fat, and carbohydrate in this portion. The present writer hopes soon to publish such a table, from which representative items on the following page are extracted.

The chief advantage of this method of expressing food values is that it lends itself readily to geometrical representation. The difficulty in the practical use of most tables of food values is that they cannot be visualized.

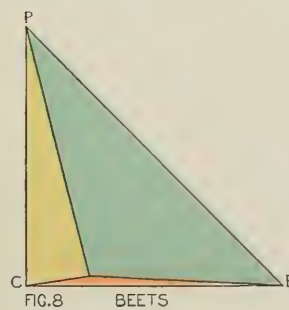
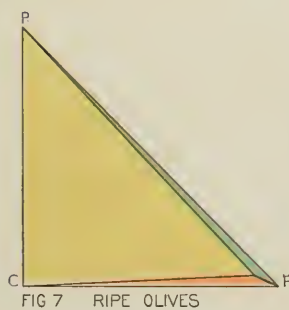
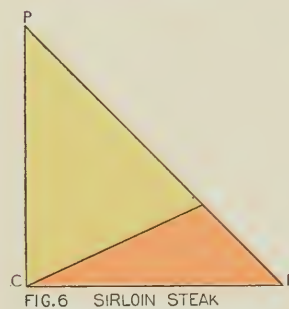
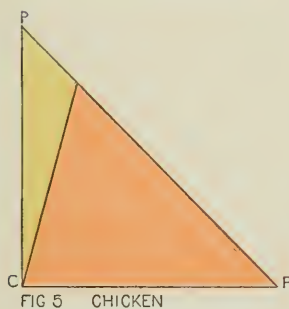
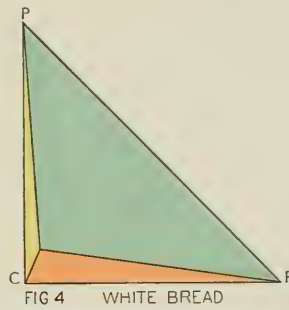
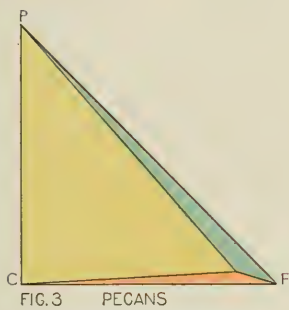
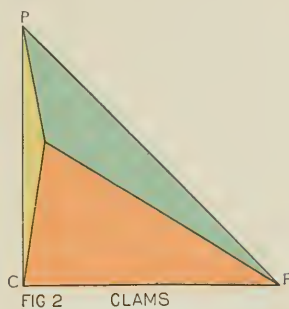
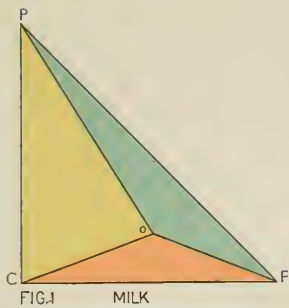
In order to show to the eye the character of the different foods (Fig. 1), let the area of the triangle *CFP* represent 100 calories of any particular kind of food, — for example, milk, — and the three triangles into which the whole triangle is subdivided, namely, *COF*, *COP*, and *FPO*, represent the constituents of proteid, fat, and carbohydrate respectively, each being measured, *not by weight but by calories*. Red best represents proteid, since proteid produces red flesh and blood, and also since red meat is a common source of proteid; yellow, the color of butter, may best represent fat; and green may be used to represent carbohydrate, since most green vegetables consist chiefly of carbohydrates.

In such a representation the triangle may be of any shape, but for our present purposes it is convenient that it should be a right triangle, and that the two sides about the right angle should be equal. The advantage of this particular form is that it enables us to make use of ordinary square-checked plotting paper.

Name of food and "portion" roughly estimated.	Weight containing 100 calories.		Proteid.	Fat.	Carbo-hydrate.	Total.
	oz.	gram.				
Almonds, a dozen . . . .	0.53	15	13	77	10	100
Apple pie, a third of a piece	1.3	36	5	32	63	100
Bacon, ordinary serving . .	0.53	15	6	94	..	100
Bananas, one large. . . .	3.5	98	5	5	90	100
Bread, a large slice . . . .	1.3	37	13	6	81	100
Butter, an ordinary pat . .	0.44	13	0.5	99.5	..	100
Cheese, an ordinary piece .	0.77	22	25	73	2	100
Chicken, an ordinary piece .	3.2	90	79	21	..	100
Cream, ordinary serving . .	1.7	49	5	87	8	100
Beef sirloin, a small piece .	1.4	40	31	69	..	100
Eggs, one large . . . . .	2.1	60	32	68	..	100
Grapes, one bunch . . . . .	4.8	140	5	15	80	100
Oysters, a dozen . . . . .	6.8	190	49	22	29	100
Potatoes, one . . . . .	3.6	100	10	1	89	100
Whole milk, two-thirds glass	4.9	140	19	52	29	100
Sugar, five teaspoons . . . .	0.86	24	....	....	100	100

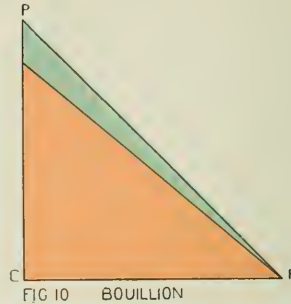
Figs. 1 to 4 indicate the composition of proteid, fat, and carbohydrate of certain typical foods: milk, in which all three elements are well represented; clams, which consist chiefly of proteid; pecans, which consist chiefly of fat; bread, consisting chiefly of carbohydrate. Figs. 5 to 10 represent still other types, namely, chicken and beefsteak, both deficient in carbohydrate; olives and beets, both deficient in proteid; and figs and commercial bouillon, both deficient in fat.

The reader will now be asking himself how to locate the point *O* in order to draw the three constituent triangles for any given food. An inspection of the diagrams will show that the areas of the con-





stituent triangles will vary according to the position of their common point  $O$ . If the point  $O$  is near  $C$  (see Fig. 1), the food consists chiefly of carbohydrates, hence  $C$  may be called the carbohydrate vertex. If  $O$  is near  $F$ , the food consists chiefly of fat, hence  $F$  may be called the fat vertex; if near  $P$ , chiefly of proteid, hence  $P$  may be called the proteid vertex. The percentage of proteid varies exactly in proportion to the elevation of the point  $O$  above the base

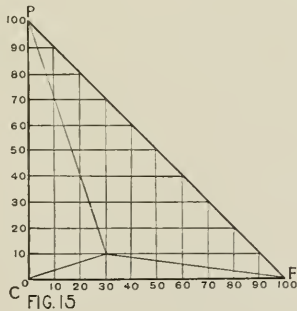
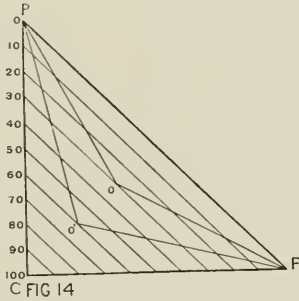
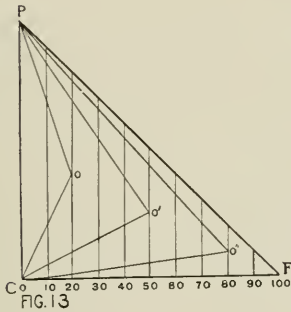
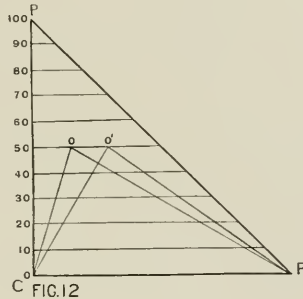
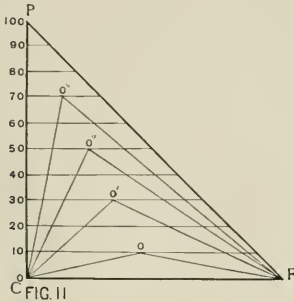


line  $CF$ . For instance, if it is at  $O''$  on the line marked 50 per cent (Fig. 11), which is half-way between the base  $CF$  and the vertex  $P$ , the triangle  $CO''F$  is just half of the triangle  $CFP$ , and consequently the proteid will constitute 50 per cent of the food value. That the one triangle is half the other is evident, because the proteid triangle  $CO''F$  and the whole triangle  $CFP$  have the same base,  $CF$ , but the altitude of the proteid triangle is just 50 per cent of the altitude of the whole triangle. Since the area of a triangle is equal to half the product of its base by its altitude, it follows that triangles having the same bases have their areas in proportion to their altitudes.

In the same way the proteid triangle  $COF$  (Fig. 11) must consist of just 10 per cent of the total triangle  $CFP$ , because its vertex  $O$  is 10 per cent as high above  $CF$  as is the vertex  $P$  of the whole triangle above  $CF$ . For similar reasons,  $CO'F$  (Fig. 11) is 30 per cent of the total, and  $CO''F$ , 70 per cent.

It is to be observed that the altitude of the triangle, or the *height* of the point  $O$  above the line  $CF$ , alone determines the size of the proteid angle. Thus, the triangle  $CO'F$  (Fig. 12), which has the same altitude as the triangle  $COF$ , has the same area, namely, half of the whole triangle  $CFP$ . It is also clear that the amount of proteid varies in exact proportion to the elevation of the point  $O$  above  $CF$ . *High* proteid is represented by a point  $O$  *high* above  $CF$ ;

*low* proteid by a point relatively *low* in the triangle. Precisely the same explanations apply to fat. The area of the fat triangle depends on the position of the point *O* right or left, and varies in exact propor-



tion of the distance *O* from the vertical line *CP*. Thus (Fig. 13), the fat triangle *COP* is 20 per cent of the total triangle, because *O* is on a line 20 per cent distant to the right of *CP*. Again, the fat triangle *CO'P* is 50 per cent of the total, and *CO''P*, 80 per cent. Finally, the carbohydrate triangle varies in proportion to the distance of the point *O* from the side *FP*. Thus (Fig. 14), the carbohydrate

triangle  $FOP$  is 30 per cent of the total triangle, because  $O$  is on a line 30 per cent away from  $FP$  toward the opposite vertex  $C$ . Again,  $FO'P$  is 60 per cent of the total triangle.

From this description it is clear that, by ruling the original triangle by lines parallel to its three sides, as shown in the diagrams, it is possible to read off the percentage of proteid, fat, and carbohydrate. Practically, it will not be necessary to rule the lines parallel to the hypotenuse  $FP$ . It will suffice to use square-checked paper which gives lines parallel to  $CF$  and  $CP$ . These lines will enable one to see the exact percentage of fat and proteid, and from these the carbohydrate can be easily found, it being necessarily the remainder out of the total 100 calories. Thus (Fig. 15) the location of the point  $O$  shows that 10 per cent is fat and 30 per cent proteid. From this it follows that the remainder, or 60 per cent, must be carbohydrate.

Since the areas of the three constituent triangles depend upon the position of the point  $O$ , it is clear that the mere *position* of this point may be used, unaided by the three constituent triangles, to indicate the percentage composition of any food. It is therefore not really necessary to draw the lines  $OC$ ,  $OF$ ,  $OP$ . Hence, many different foods may be represented on the same diagram, each by a single point. In (Fig 16) the various foods which were represented in the separate diagrams above are all thus represented on the same diagram.

It is now evident that, by means of a table of foods, an example of which is given above, it is easy to locate a point to represent a food, by a method quite analogous to locating a point on a map when its latitude and longitude are known. It is simply necessary to lay off a point  $x$  on  $CF$  (Fig. 17) to the right of  $C$ , a distance represented by the percentage of fat, and lay off a line vertically from this point, representing the percentage of proteid. The upper end  $O$  of this line will be the point required.

The geometrical representation which has been given leads to an easy method of determining the constituents of *combinations* of different foods. For instance, if we desire to ascertain the point representing a chicken sandwich, composed of bread and chicken in equal proportions (by *calories*, not by weight), we may do so by taking the middle point of the straight line (Fig. 17) joining the point which represents the two ingredients, bread and chicken. That this geometrical solution is correct may be better understood if we first make an arithmetical demonstration. Let one "standard portion" of bread be combined with one "standard portion" of chicken, which,

from the table, means 1.3 ounces of bread and 3.2 ounces of chicken. This will make the chicken sandwich weigh, all together, the sum of these, or 4.5 ounces, and this sandwich will contain 200 calories. Half of it will therefore be a "standard portion" of chicken sandwich, and will weigh 2.25 ounces, *i. e.*, the *average*, of the weight of the standard portions of the bread and chicken separately.

In the same way it may be shown that the percentage of proteid in the chicken sandwich is the average of the percentages of proteid in the bread and the chicken. If the standard portion of bread contains 13 calories of proteid, and the standard portion of chicken 79, when the two were combined the total proteid would be the sum, or 92 calories. But this would be the amount of proteid in two standard portions. In one standard portion there would be therefore half this amount, or 46 calories, which is the average of 13 and 79.

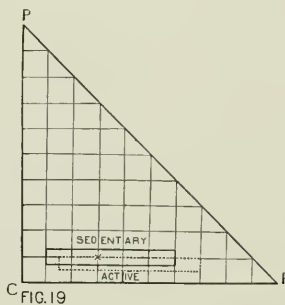
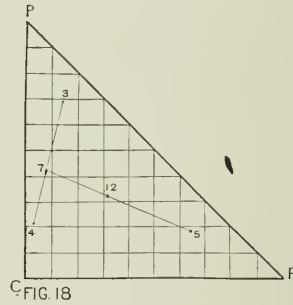
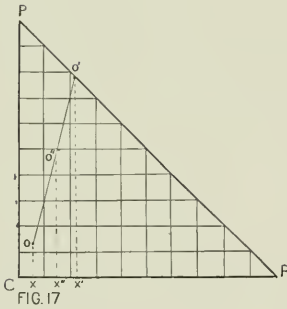
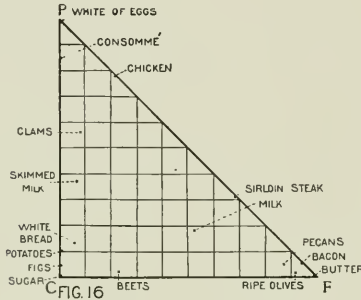
Since the same reasoning applies to the other elements,—fat and carbohydrate,—the process of combining the bread and chicken evidently consists merely in averaging the numbers which apply to them both, and this may be shown in the following table:

	Weight of standard portion.	Proteid.	Fat.	Carbohydrate.
Bread . . . . .	1.3	13	6	80
Chicken . . . . .	<u>3.2</u>	<u>79</u>	<u>21</u>	<u>0</u>
Chicken sandwich, av. .	2.25	46	14	40

Hence the geometrical problem consists simply in finding a point  $O''$  (Fig. 17) such that the proteid, fat, and carbohydrate represented by it are respectively the averages of the proteid, fat, and carbohydrate represented by  $O$  and  $O'$ . The point that satisfies these requirements is the point midway on the straight line connecting  $O$  and  $O'$ . Thus the vertical line  $O''X''$  is the average of the heights of  $OX$  and  $O'X'$ .<sup>1</sup> The same reasoning applies to the fat and the carbohydrate. It follows that  $O''$ , the middle point of the straight line  $OO'$ , correctly represents the food proportions of the chicken sandwich. If the bread and chicken were combined in other proportions, the point representing the chicken sandwich would be represented by some other point on the straight line  $OO'$ . For instance, if one "standard portion" of bread were combined with half a "stand-

<sup>1</sup> "The line ( $O''X''$ ) drawn parallel to the two parallel sides ( $OX$  and  $O'X'$ ) of a trapezoid ( $OXX'O'$ ) through the middle of one of the non-parallel sides ( $OO'$ ) is equal to half of the sum of the parallel sides." See *e. g.*, PHILLIPS and FISHER'S Geometry, p. 68.

ard portion" of chicken, the point  $O''$  representing chicken sandwich would be situated twice as near the bread ( $O$ ) as the chicken ( $O'$ ). The proof of this is similar to that given for the case of equal portions.



In general, then, when any two foods are combined, the food proportions of the combination will be represented by a point on a straight line joining the two points which represent the two respective ingredients, and will divide the distance between them in the inverse ratio of the ingredients (measured, of course, by *calories*, not

by weight). The point  $O''$  is what is called in physics the "centre of gravity" of the points  $O$  and  $O'$ , each "weighted" in proportion to the calories contained in the two ingredients.

When three foods are combined, the point representing the combination is in like manner the "centre of gravity" of the three, and may be found by first obtaining the centre of gravity of two, and then obtaining the centre of gravity of the point thus obtained, and the third. Thus if, as in Fig. 18, we have three points representing respectively 3, 4, and 5 calories of three separate foods, shown by the attached numbers 3, 4, and 5, the point representing the combination may be found by joining the points labelled 3 and 4, and finding their centre of gravity, 7, situated nearer the point 4 than point 3, and dividing the line between them in the ratio of 3 to 4. The first two points, 3 and 4, may be considered as concentrated at 7 with their combined weight, 7. We then find the centre of gravity of this new point 7 and the remaining point, 5. The centre of gravity of this point 7 and point 5 will be a point, 12, on the straight line between them, situated nearer the 7 than the 5, and dividing the distance between in the ratio of 5 to 7. At point 12 the whole combination of 12 portions may be considered to be concentrated. It is evident that we could find the centre of gravity of the same three points by combining them in a different order, but the result would be the same.

In precisely the same manner we may obtain the point representing the combination of any number of different foods simply by taking the centre of gravity of the points representing the respective foods, each being weighted in proportion to the calories or standard portions which enter into the combination. In this way it is possible to obtain very quickly the point representing the daily diet, no matter how many separate ingredients may enter into it. It is only necessary to indicate each ingredient by a point on the triangle, and the number of "portions" of it used, by a number placed opposite that point, and then to combine them by the rules just given.

Since the resultant point is the centre of gravity, it is evident that it can be obtained by mechanical as well as by geometrical methods. For this purpose a mechanical diet indicator has been devised, as shown in Figs. 20, 21, 22, 23.

The essential feature of this apparatus is a card on which is drawn the right-angled triangle with which the reader has become familiar. Points on this card may be located to represent the various foods



employed. These points may be easily found from tables like the sample above given. Points for the most common foods may be already printed upon it. At points representing foods eaten, pins with heavy heads are thrust through the cardboard (Fig. 20), the weight of each representing one "standard portion." Similar pins

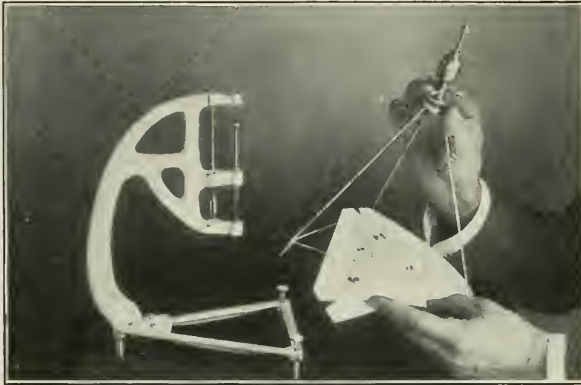


FIGURE 20.

to represent half and quarter portions are also provided. When these pins are placed, the total ration which has been consumed is easily found simply by counting the portions thus represented. For instance, if there are 15 pins representing "standard portions" and 10 pins representing half "portions" (and therefore 5 full "portions"), the total ration is 20 "portions," or 2000 calories. In order to find the percentages of proteid, fat, and carbohydrate in this ration, it is only necessary to obtain the centre of gravity of all the pins. For this purpose the card is placed in a basket (Fig. 20) and suspended on a standard (Fig. 21), so that the centre of gravity of the pins may assume a position vertically under the point of support (Fig. 22). It is necessary, of course, that the influence of the weight of the cardboard and the basket containing it shall be entirely eliminated. This is accomplished by the construction of the basket. Its centre of gravity (when containing the cardboard *without* pins) exactly coincides with the point of support. Hence, when the basket with the card (but without pins) is placed on the point of support, it tends neither to swing (which would imply stable equilibrium) nor to be top-heavy (which would imply unstable equilibrium), but is in "neutral" equilibrium, and will remain at any angle at which it is placed

When, now, the weighted pins are placed on the cardboard, their influence causes the basket to seek stable equilibrium, and the centre of gravity of the pins will then find its position vertically under the point of support. The position of this centre of gravity is easily indicated on the card by means of a vertical pricker (Fig. 22) which is so arranged that it may be pressed upon the card. Thus, almost instantaneously, the centre of gravity is found. The total time consumed in placing the pins, adjusting the card and basket, and finding the centre of gravity, is found to be about half the time required for solving the same problem by the geometrical method, about one-quarter of the time required if the method of "calories per ounce" is used, and about one-tenth of the time required if the method of "weight per cent" is employed.



FIGURE 21.

It is thus seen that the method of percentage of calories is much more rapid in application, whether applied geometrically or mechanically. It may also be applied arithmetically if desired. When the centre of gravity is found, whether by the arithmetical, geometrical, or mechanical plan, one may see at a glance whether the ration is a "well balanced" one or not. For instance, let us assume as the normal ration for sedentary persons the average of the five sedentary men participating in the experiments of Professor Chittenden at Yale in 1904. The average weight of those persons was 140 pounds, and the average calories consumed 2,100, of which 10 per cent were in the form of proteid, 28 per cent in the form of fat, and the remainder of the 100, carbohydrate.

It must be remembered, however, that Professor Chittenden's experiments were made chiefly for the purpose of determining the minimum nitrogen or proteid requirements, and that this element alone was obtained by chemical analysis, while the calories were simply estimated from tables. The average standard thus obtained

should be described as a *sedentary minimum* for healthy persons of a body weight of 140 pounds, and exact only for proteid. The proteid requirement varies in direct proportion to the body weight. It may be found by multiplying the body weight in pounds by

one and one-half. It does not vary appreciably with the activity. The total calories, on the other hand, will vary with the activity, and for the non-sedentary person would be considerably larger than the minimum just given. Moreover, the amount of proteid required is subject to some individual variation, which, judging from the 35 persons in the Chittenden experiment, would seldom be outside of the range of 1.3 and 1.7 calories per pound of body weight. The percentages of fat and carbohydrate are subject to much wider variations, the limits of which have never been, and perhaps can never be, definitely determined.

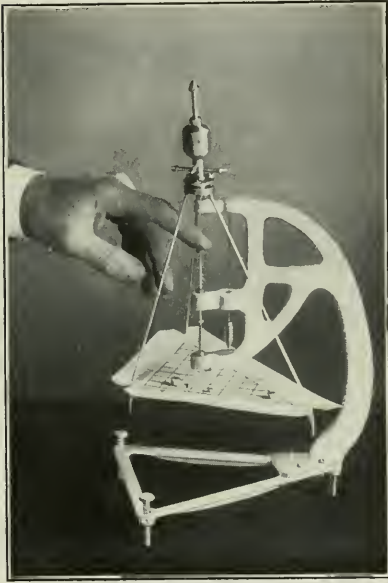


FIGURE 22.

With the provisos and reservations just made, we may now indicate the allowable range in the centre of gravity of diet for sedentary persons by the rectangle indicated as "sedentary" in one of the previous diagrams (Fig. 19). On the same diagram is indicated another rectangle showing the allowable range for a more active person requiring 50 per cent more calories. Since his proteid requirement depends only on the weight and will not appreciably increase with activity, it follows that an increase of 50 per cent in total calories implies a decrease in the *percentage* of proteid. Consequently the rectangle indicating the allowable range for active persons is somewhat lower than for sedentary persons. In other words, active persons should choose foods of rather lower proteid content than sedentary persons. This result is, of course, the direct opposite of the traditional belief, based on the old idea that working energy comes from proteid.

The mechanism in which the cardboard triangle is held is so

arranged that when the centre of gravity falls at the point marked with a  $\times$  (Fig. 19) indicating the average daily food proportions for sedentary persons, the cardboard will be exactly horizontal. If the centre of gravity is at some other point, the cardboard will incline, showing that the ration is not "balanced." If the proteid vertex is tipping down, it indicates an excess of proteid; if the fat vertex, excess of fats; if the carbohydrate vertex, an excess of carbohydrates.

It is now evident that, by means of the mechanism and the rectangles marked upon the card, it is a simple matter to discover the conformity or non-conformity of a diet to any given standard. It is equally simple to correct discrepancies thus found, and, if need be, the corrections may be made in advance and the balanced rations prescribed. One advantage, however, of the present method is that, whether the object is merely to record what is consumed or actually to prescribe, the patient need

not be annoyed by having scales at the table. All weighing may be done in the kitchen and the food served in "standard portions" or in known multiples or fractions of such portions. It is only necessary that the subject or some one else shall report exactly what part of the various portions served have been consumed. The subject need not even know what are the food values of the portions served to him, and thus the introspection and trepidation which often defeat the very aim of food prescription may be avoided. Again, for those who desire to keep a record of their dietary and can do so without disadvantage, the method here given has the great advantage that when it is once made familiar it can be used roughly almost without any apparatus. One soon becomes able to recognize the quantities of the ordinary foods which contain 100 calories, and to hold in memory the position on the triangle where these foods are indicated. Very little thought will then enable him to judge how many "stan-

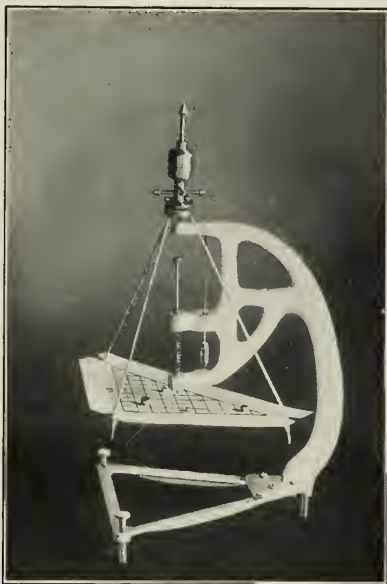


FIGURE 23.

dard portions" he has consumed at a meal, and also whether their centre of gravity falls within the rectangles indicated.

The chief use for the method, however, will doubtless be found in sanatoria<sup>1</sup> where it is important to record and correct the diet of a large number of patients. There is now a movement among the various sanatoria, particularly those dealing with tuberculosis, toward a more accurate dietetic treatment of their patients. However, as yet no uniform standard either of quantity or proportions has obtained any general recognition. The writer intends in another place to show these discrepancies between various consumptive sanatoria. The present paper aims merely to indicate a general method which may be applied to any standard whatever.

<sup>1</sup> Several institutions to which the writer has shown the device have already adopted it. Among them is the large sanatorium at Battle Creek, Michigan, from which the instrument may be obtained. Others are also free to construct it, as there is no patent.



## A STUDY OF PARA-AETH-OXY-PHENYL-CAMPHORYL- IMID (CAMPHENAL) AS AN ANTIPYRETIC.

BY E. M. HOUGHTON.

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SOME months ago my attention was called to a new chemical compound which the discoverers (A. H. C. Heitmann and E. C. Clemmensen) believed would be of value as an antipyretic. This substance, which they have provisionally called "Camphenal," is a condensation product of camphoric acid and para-amido-phenetol.

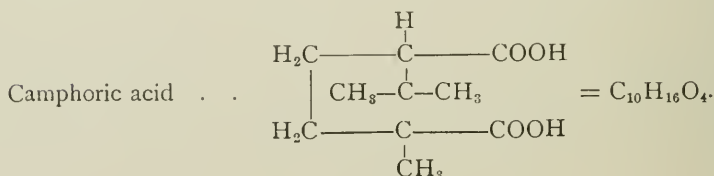
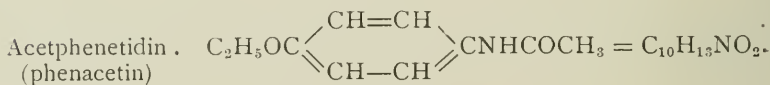
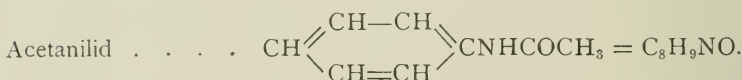
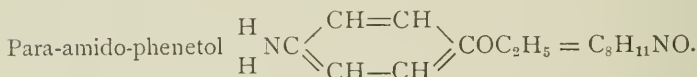
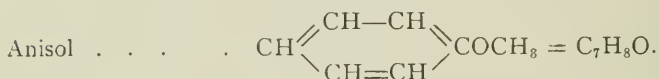
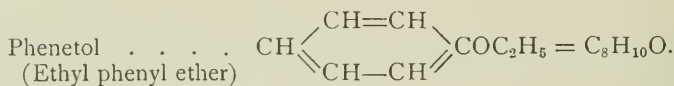
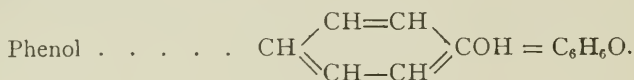
Camphenal occurs in silky, colorless needles, odorless, tasteless, and permanent in the air; is sparingly soluble in cold water, more soluble in hot water, and very soluble in hot diluted alcohol, from which it crystallizes on cooling; is very soluble in the organic solvents, as alcohol, acetone, benzole, ether, chloroform, etc. Acids and alkalis do not affect its solubility, and it is very stable, not being split up by these reagents in hot concentrated form. It melts at  $119^{\circ}$  C.

A consideration of the chemical formula of this substance leads one to believe that it should possess the pharmacologic properties that would be of value for therapeutic purposes, especially as an antipyretic.

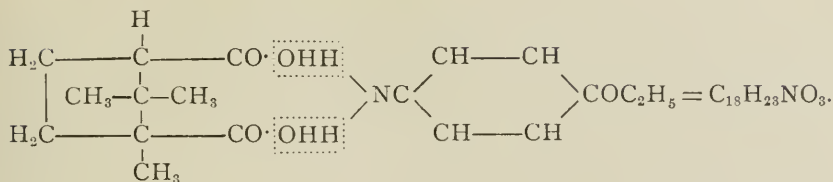
Many anilin derivatives have been employed as antipyretics, the best known being acetanilid and acetphenetidin (phenacetin), which are now pharmacopœial. Both of these and allied products have been the subject of frequent discussions and are believed by many physicians to be more or less dangerous because of their untoward action upon the heart. It is not my purpose to consider in detail whether this belief is well grounded on fact, but assuming that it be true, it was thought that such depressing action might possibly be due to the acetyl group ( $\text{CH}_3\text{CO}$ ), as it is contained in both substances. Since acetphenetidin differs from acetanilid in containing the aeth-oxy group and is looked upon as much the safer product, it was thought best to attempt to replace its acetyl group ( $\text{CH}_3\text{CO}$ ) with some sub-



stance which had a stimulating action upon the central nervous system and heart. After considerable experimentation it was found that camphoric acid and para-amido-phenetol could be caused to unite under certain conditions, two molecules of water being split off in the reaction. A brief consideration of the following formulæ will show the chemical relation of this new compound to anilin, acetanilid, phenacetin, etc.:



Para-aeth-oxy-phenyl-camphoryl-imid.



It will be observed that in acetanilid one H of the amido group has been replaced by an acetyl group. Acetphenetidin likewise has one amido H replaced by an acetyl group, but also contains an aethoxy group in the para position in addition, thus bearing a strong resemblance not only to acetanilid but also to para-amido-phenetol. In fact, phenacetin may be looked upon as a connecting link between these two substances.

Pharmacologists have not been able to offer an entirely satisfactory explanation to account for the depression of the heart and collapse which sometimes occur, or the peculiar action of these drugs upon the heat centres that render them of so much value in therapeutics, and my experiments were not designed primarily to answer the question, but to determine the specific antipyretic value of camphenal.

The camphor group, including camphoric acid, generally is regarded as of value in collapse because it stimulates the central nervous system, including the circulatory centres and the heart muscle itself. It was believed that the new compound, from its chemical formula as determined by combustion, might prove less depressing and still possess valuable antipyretic properties. Accordingly, a series of experiments was undertaken upon animals to show its toxic action and its value as an antipyretic, comparing it with acetphenetidin. Owing to the great insolubility of the drug, it was found impossible to study its action upon frogs or when given intravenously or subcutaneously to larger animals. Dogs were chosen as being most suitable for the work. The influence of camphenal as an antipyretic compared with acetphenetidin can be best studied under three heads. (See tables Nos. 1, 2, 3, 4, and 5 and Tracings a, b, c, d, and e.) In some cases, in order to shorten the tables, observations have been omitted where the results did not differ from the last record.

## I. ACTION UPON NORMAL DOGS.

Several normal healthy dogs were given large quantities of camphenal and acetphenetidin in gelatin capsules, by placing the capsules far back in the mouth and compelling the animal to swallow them. Rectal temperature was usually taken every hour, beginning two hours before the drug was given and continuing for several hours after. At the time the temperature was taken respiratory movements and heart beats were counted. As the results obtained were somewhat contradictory, I shall not discuss them.

TABLE I.

Dog No. 22. Weight, 15 kilos (see Fig. 1).

Time.	Temperature.	Remarks.
8.00 A.M.	102.6°	Normal.
9.00 "	101.6°	"
10.00 "	101.1°	Given 2 gm. camphenal in capsules.
11.00 "	100.6°	
12.00 M.	100.4°	
1.00 P.M.	100.5°	
2.00 "	101.2°	
3.00 "	100.4°	
4.00 "	100.8°	

Dog No. 20. Weight, 8.5 kilos.

5.30 A.M.	101.8°	Normal.
7.00 "	102.2°	"
9.15 "	102.2°	Given 1 gm. camphenal in capsules.
10.30 "	103.2°	
12.00 M.	103.5°	
2.00 P.M.	103.8°	
2.15 "	....	Given 1 gm. acetphenetidin in capsules.
3.15 "	103.0°	
4.00 "	102.0°	
5.00 "	101.6°	
7.00 "	101.7°	
8.00 "	102.0°	

It will be observed that camphenal, when given to these two dogs in reasonably sized doses, did not produce any appreciable influence on the temperature. Acetphenetidin did produce considerable lowering in temperature in dog No. 20, but the results, even with this drug when given in small doses to normal dogs, did not produce constant antipyretic effects.

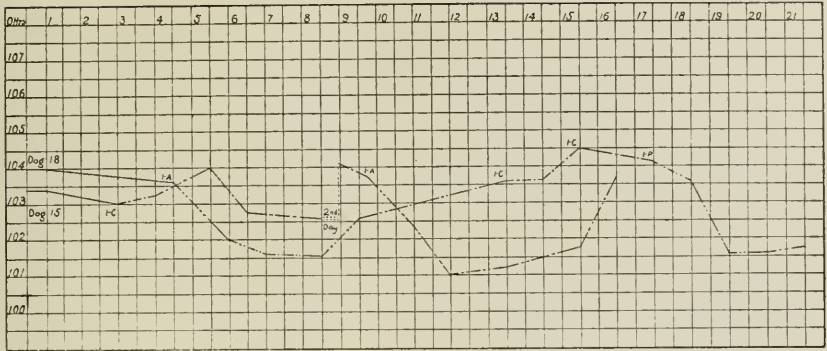


FIGURE 1.—Dog No. 18, weight 12.5 kilos; experiment began at 5.30 A.M. Dog No. 15, weight 12.5 kilos; experiment began at 9.00 A.M. ——— normal. ——— camphenal. ——— acetphenetidin and phenacetin.

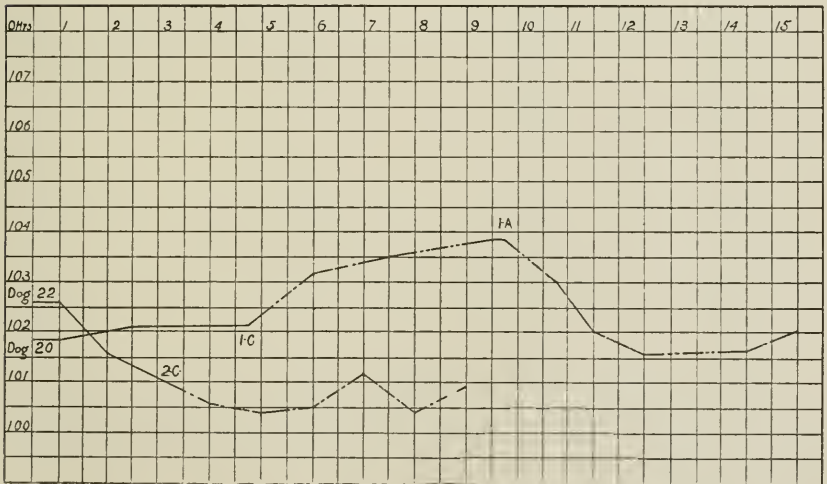


FIGURE 2.—Dog No. 22, weight 15 kilos; experiment began at 8 A.M. Dog No. 20, weight 8.5 kilos; experiment began at 5.30 A.M. ——— normal. ——— camphenal para-aeth-oxy-phenyl-camphoryl-imid. ——— acetphenetidin.

II. ANTIPYRETIC EFFECT UPON DOGS SUFFERING FROM  
SO-CALLED "DOG DISTEMPER."

TABLE II.

Dog No. 18. Weight, 12.5 kilos (see Fig. 2).

Time.	Temperature.	Remarks.
5.30 A.M.	104.0°	Before drug was given.
9.00 "	103.6°	Before drug was given. Given 1 gm. acetphenetidin in capsules.
9.30 "	103.0°	
10.30 "	102.1°	
11.30 "	101.7°	
1.00 P.M.	101.6°	
2.00 "	102.6°	
6.00 "	103.7°	Given 1 gm. camphenal in capsules
7.00 "	103.7°	
8.00 "	104.5°	Given 1 gm. camphenal.
10.00 "	104.2°	Given 1 gm. phenacetin.
11.00 "	103.6°	
12.00 Night	101.6°	
2.00 A.M.	101.6°	
3.00 "	101.8°	
Discontinued		

Dog No. 15. Weight 12.5 kilos.

9.00 A.M.	103.4°	Before drug was given.
11.00 "	103.0°	Given 1 gm. camphenal in capsules.
12.00 "	103.3°	
1.30 P.M.	104.0°	
2.30 "	102.8°	
4.30 "	102.6°	
Next day.		
8.30 A.M.	104.2°	
9.15 "	103.8°	Given 1 gm. acetphenetidin in capsules.
10.30 "	102.4°	
11.30 "	101.0°	
1.30 P.M.	101.2°	
3.30 "	101.8°	
4.30 "	103.7°	

These experiments show conclusively that acetphenetidin is much more constant in producing an antipyretic action than camphenal where there is an abnormally high temperature produced by an infectious disease.

As another means of checking these results and showing the antipyretic value of the two substances, several normal dogs were given each an injection of 0.05 c.c. of diphtheria toxin, the L + dose of which was about 0.0028 c.c.

III. ACTION OF DIPHTHERIA TOXIN UPON THE TEMPERATURE OF NORMAL DOGS.

TABLE III. (See Fig. 3.)

Time.	Temperature.	Remarks.
8.00 A.M.	102.5°	Normal.
9.00 "	101.6°	"
9.30 "	...	Injected subcutaneously 0.05 c.c. diphtheria diluted to 1 c.c. with saline solution.
11.00 "	101.1°	
3.30 P.M.	103.2°	
5.30 "	106.7°	
Next day.		
7.30 A.M.	103.5°	Dog did not eat during the night, and appears to be very sick. Temperature gradually fell during the day.
5.00 P.M.	102.5°	
Third day.		
9.00 A.M.	100.1°	Animal very sick; died during the third night.
5.00 P.M.	98.0°	
Dog No. 2. Weight, 10 kilos.		
8.00 A.M.	102.1°	Normal.
9.00 "	101.2°	"
9.30 "		Injected 0.05 diphtheria toxin diluted as above.
11.00 "	100.5°	
3.30 P.M.	102.8°	
5.30 "	105.2°	Animal is very sick.
Next day.		
7.30 A.M.	103.5°	
9.00 "	104.6°	Animal is very sick.
5.00 P.M.	101.0°	
Third day.		
Temperature falls to	99.5°	Animal is very sick.
Fourth day.		
9.30 A.M.	100.8°	Given 4000 units of antidiphtheric serum.
6.00 P.M.	100.7°	" " " " "
Fifth day.		
8.00 A.M.	101.1°	Given 3000 units antidiphtheric serum.
10.00 "		Dog died.

Experiments on these two dogs and several others similarly treated showed that the diphtheria toxin was followed by a slight temporary fall in temperature, but at the end of about eight hours there was a rise of several degrees and then during the next week the temperature fell to subnormal, the animal dying of typical toxæmia. In the later stages of the toxæmia, anti-diphtheric serum does not prevent death.



IV. INFLUENCE OF ACETPHENETIDIN UPON THE TEMPERATURE OF DOGS RECEIVING DIPHTHERIA TOXIN.

TABLE IV.

Dog No. 4. Weight 7.5 kilos (see Fig. 4).

Time.	Temperature.	Remarks.
8.00 A.M.	101.5°	
8.30 "		Injected 0.05 c.c. diphtheria toxin.
3.00 P.M.	102.4°	
4.00 "	103.0°	Given 1 gm. acetphenetidín per stomach in capsules.
5.00 "	102.5°	
6.00 "	101.8°	
9.00 "	103.0°	

Dog No. 5. Weight 11.7 kilos.

8.00 A.M.	102.6°	
8.30 "		Diphtheria toxin. 1 gm. acetphenetidín per stomach.
9.00 "	102.6°	
10.00 "	102.4°	
11.00 "	101.6°	
2.00 P.M.	102.2°	
3.00 "	104.0°	Given 1 gm. acetphenetidín.
4.00 "	103.0°	
5.00 "	102.2°	
9.00 "	104.0°	

These two experiments show very clearly that acetphenetidín does act as a powerful antipyretic.

V. INFLUENCE OF CAMPHENAL UPON THE TEMPERATURE OF DOGS RECEIVING DIPHTHERIA TOXIN.

TABLE V.

Dog No. 8. (See Fig. 5.)

Time.	Temperature.	Remarks.
7.30 A.M.	102.2°	Normal.
8.30 "	102.3°	Injected 0.05 diphtheria toxin subcutaneously.
9.30 "	101.0°	
1.30 P.M.	102.4°	
2.30 "	104.0°	Gave 1 gm. camphenal per stomach in capsules.
4.30 "	104.6°	
8.00 "	104.0°	

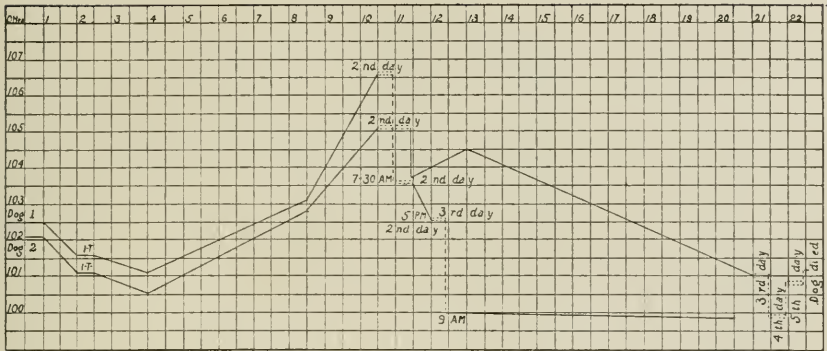


FIGURE 3.—Action of diphtheria toxin on temperature of normal dogs. Dog No. 1, weight 12.5 kilos. Dog No. 2, weight 10 kilos.

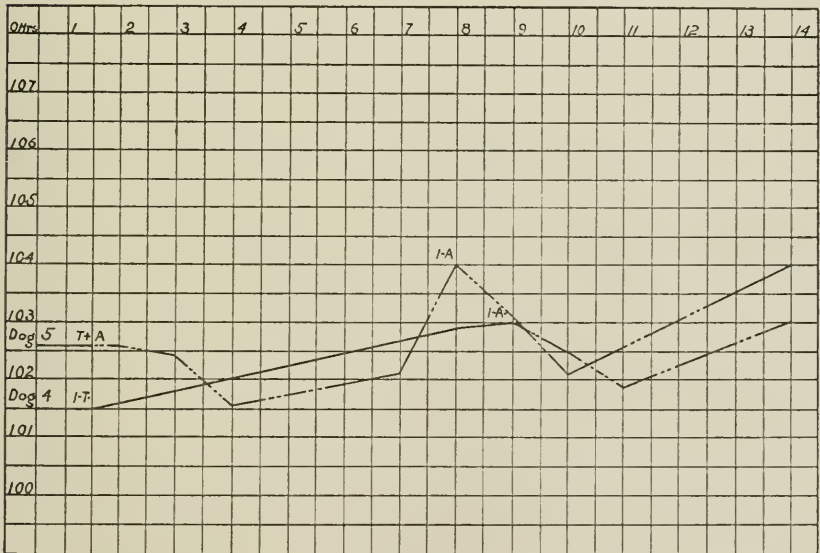


FIGURE 4.—Dog No. 5, weight 11.7 kilos; experiment began at 8 A.M. Dog No. 4, weight 7.5 kilos, experiment began at 8 A.M. ——— normal and diphtheria toxin = T. - - - - acetphenetidin = A.

TABLE V. (continued).

Dog No. 10. Weight, 7.5 kilos.

Time.	Temperature.	Remarks.
8.00 A.M.	101.9°	Normal.
9.00 "	101.6°	"
10.00 "	102.4°	Injected 0.05 c.c. diphtheria toxin. Gave 1 gm. camphenal per stomach.
11.30 "	101.9°	
1.00 P.M.	103.0°	Gave 1 gm. camphenal.
2.00 "	105.6°	" " "
2.30 "	"	" " "
3.00 "	106.5°	" " "
3.30 "	"	" " "
5.30 "	106.9°	Discontinued.

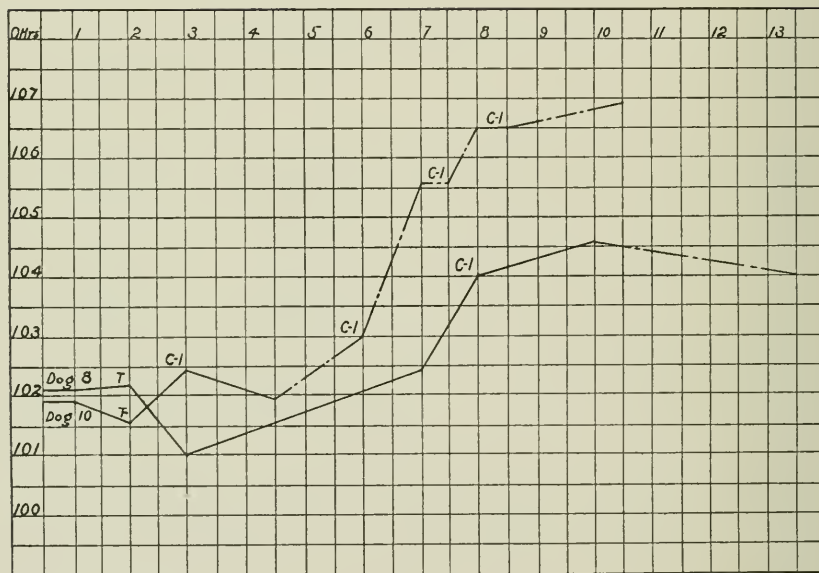


FIGURE 5.—Dog No. 8, weight 7.5 kilos; experiment began at 7.30 A.M. Dog No. 10, weight 7.5 kilos; experiment began at 8 A.M. ——— normal and diphtheria toxin = T. — - — - camphenal para-aeth-oxy-phenyl-camphoryl-imid = C. — - - — phenacetin = P.

These experiments show quite conclusively that camphenal has but slight antipyretic action as compared with acetphenetidin.

Experiments were also made upon dogs receiving diphtheria toxin, giving acetanilid instead of acetphenetidin. This also produced a

prompt antipyretic action. All dogs receiving the diphtheria toxin, whether they received any further treatment or not, died in about the usual time from diphtheria paralysis.

We may conclude from these experiments that the drug is not of value as an antipyretic, and in fact the experiments do not demonstrate that it would be of any value as a therapeutic agent. It is of interest, however, in showing that the camphoryl group cannot take the place of the acetyl group in acetphenetidin without diminishing the antipyretic property.

## SUPRARENAL TRANSPLANTATION WITH PRESERVATION OF FUNCTION

BY F. C. BUSCH AND C. VAN BERGEN.

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TRANSPLANTATION or grafting of the suprarenal glands has not hitherto yielded the same functional success that has attended similar experiments in connection with the thyroids and the pancreas. The reason apparently has been the difficulty of preservation of the medullary part of the gland which alone contains the active principle and which is functionally essential.

The first attempt of suprarenal transplantation reported was that of Canalis,<sup>1</sup> who, out of all his cases, succeeded once only in demonstrating cortical cells with suprarenal capsule in the cicatrix of the wound where the piece had been transplanted.

Langlois,<sup>2</sup> out of thirty experiments in frogs, reported eight operatively successful cases. These frogs survived destruction of other suprarenal tissue. Control frogs died. Destruction of the grafts in the grafted frogs was followed by death in six. Two survived, one dying later, at the end of twelve days, the other escaping. Doubt is thrown upon the significance of these results by Christiani.

Gourfein,<sup>3</sup> experimenting on frogs, and Boinet,<sup>4</sup> on rats, had negative results.

Hultgren and Anderson<sup>5</sup> transplanted into the dorsal musculature of cats and rabbits. The transplanted pieces became necrotic.

<sup>1</sup> CANALIS: *Internationale Monatsschrift für Anatomie und Physiologie*, 1887, iv, p. 312.

<sup>2</sup> LANGLOIS: *Thèse de la faculté des sciences, Paris*, 1897.

<sup>3</sup> GOURFEIN: *Revue médicale de la suisse romande*, 1895.

<sup>4</sup> BOINET: *Comptes rendus de la société de biologie*, March, 1895.

<sup>5</sup> HULTGREN and ANDERSSON: *Skandinavisches Archiv für Physiologie*, 1899, ix, p. 73.

Strehl and Weiss<sup>1</sup> grafted into a pocket made in the abdominal parietes. Some grafts were left free in the abdominal cavity. They also tried transplantation into vascular organs, such as the kidney, but always with the same negative result.

Poll<sup>2</sup> made an extensive histological study of transplanted pieces of suprarenal. Out of fifty-four cases, twenty-three showed a conservation of a small part of the gland. In all cases this was the cortical portion, the medulla having been entirely replaced by connective tissue.

Schmieden<sup>3</sup> reports successful transplantation of pieces of suprarenal into the kidney parenchyma of the same animal, but, so far as we are aware, has not demonstrated continuance of function by the transplanted piece in the absence of other suprarenal tissue.

Parodi<sup>4</sup> grafted adrenals from embryo rabbits into the kidney, liver, and sciatic nerves of rabbits three or four months old, and also into adult animals. The cortex of the grafted pieces became adherent; the medulla did not. After a time this was replaced by new-formed connective tissue.

H. and A. Christiani<sup>5</sup> carried on extensive transplantation experiments in rats, but with negative results, so far as survival and preservation of the medullary portion of the suprarenal grafts was concerned.

Stilling<sup>6</sup> has succeeded in demonstrating living suprarenal tissue in the testicle over three years after transplantation of the same into that organ. This consisted, however, only of the cortical portion, the medulla having disappeared. The cortical cells had not only been preserved, but had multiplied. His results apparently show the possibility of the indefinite survival of transplanted portions of the suprarenal.

The partially successful results hitherto reported have been ob-

<sup>1</sup> STREHL and WEISS: *Archiv für die gesammte Physiologie*, 1901, lxxxvi, pp. 107-121.

<sup>2</sup> POLL: *Archiv für mikroskopische Anatomie*, 1899, liv, p. 440.

<sup>3</sup> SCHMIEDEN: *Archiv für die gesammte Physiologie*, 1902, xc, p. 113; *Deutsche Zeitschrift für Chirurgie*, 1903, lxx, p. 453.

<sup>4</sup> PARODI: *Giornale della reale accademia di medicina di Torino*, 1903, p. 401.

<sup>5</sup> H. and A. CHRISTIANI: *Journal de physiologie et de pathologie générale*, 1902, iv, p. 64.

<sup>6</sup> STILLING: *Verhandlungen der deutschen pathologischen Gesellschaft*, 1903, xv, p. 122; *Beiträge zur Pathologie, Anatomie und zur allgemeine Pathologie*, 1905, xxxvii, p. 480.



tained through homo-grafting, *i. e.*, transplanting a piece of the suprarenal into some part of the same animal. The less promising field of hetero-grafting is still unexplored.

In the summer of 1902 we undertook, in our laboratory, a series of experiments in suprarenal transplantation with the hope of being able to secure functioning grafts. The rabbit was selected as the experimental animal, and the kidney, because of its vascularity, ease of access, and from the fact that suprarenal rests are often found in it, was chosen as the tissue for implantation of the grafts.

The general plan of the complete transplantation experiment may be briefly outlined as follows:

The operative procedure consists of three stages. First, one suprarenal is removed entire, and cut sagittally into three portions by two longitudinal incisions. The middle third so obtained consists of both cortex and medulla. A piece of the cortex of the corresponding kidney is then excised, cut as near the pattern of the suprarenal piece to be grafted as possible. The graft is then inserted into the wound thus made, the cut surface of the graft being applied to the cut surface of the kidney. The wound is then approximated and the graft held in place by two or more silk sutures.

Second, at a varying period after the introduction of the graft, the remaining suprarenal gland is excised.

Third, if the animal survives the second operation, at a later period the kidney containing the graft is removed. If the graft has been keeping the animal alive in the absence of other suprarenal tissue, a fatal result should follow the third operation because of suprarenal insufficiency.

The complete experiment, as outlined above, has been performed in one case which is described in the protocols that follow. Besides this case, and in the light of it, we believe that we have obtained functioning grafts in other cases.

Thirty-one rabbits and one dog have been experimented upon. A number of animals were lost in the early operations because of various errors in technic. Among these may be mentioned the method of anesthesia and narcosis. Morphine alone was at first employed, from 6 to 12 centigrams for a three or four pound rabbit. Later, the dose of morphine was reduced and ether was used as an anesthetic. In attempting to remove the gland entire, there is considerable danger of wounding the inferior vena-cava or the renal vein with one or the other of which the gland is in close relation. This accident has oc-

curred in several instances. In two cases the kidney incision was too deep, opening into the pelvis, with subsequent extravasation of urine. In some instances the animals died from shock shortly after the primary operation. In other instances a similar result followed the second operation.

Generally the grafts were made from the left suprarenal into the left kidney, work being done through a mid-abdominal incision, since the left suprarenal is more accessible by this route. For the same reason the right suprarenal has been removed extra-peritoneally by way of the dorsal route.

Careful search was made for stumps remaining after excision of the glands and for accessory suprarenals. Microscopical examination of the grafted tissues was also made, except in those instances where the whole of the graft was extracted for possible demonstration of blood-pressure raising principle.

#### PROTOCOLS OF EXPERIMENTS.

In the following account, protocols of those experiments in which the animal died immediately after the primary operation as well as those in which death occurred after the second operation because of suprarenal insufficiency, resulting from necrosis of the graft, have not been recorded. As a rule, it was found that in those animals where death occurred after the removal of the second suprarenal, the graft had become necrotic. In these cases, also, the second suprarenal removed had undergone marked hypertrophy. This was not the case where living grafts were obtained.

**Case I.** — June 16, 1902. Large Belgian hare. Morphine sulphate, 9 cgm ; ether anesthesia. Right suprarenal removed through dorsal route. Cut into thirds, longitudinally. Middle third implanted in cortex of right kidney. Kidney wound brought together over graft and closed by three silk sutures. Fascia and skin united by two rows of continuous silk sutures. Rabbit made an uneventful recovery.

June 30, 1902, killed rabbit. *Autopsy.* — Right kidney firmly adherent to posterior abdominal wall. At this point a cheesy mass about the size of a pea was found connected with the kidney incision. Section through the abscess, kidney wound, and middle of graft showed the latter well imbedded in kidney tissue, of normal color and appearance, and unconnected with the abscess.

*Microscopic examination.* — Tissues hardened in formalin and imbedded in collodion. Sections stained with hemotoxin and eosin.

Graft shows as a bean-shaped segment entirely surrounded by kidney substance. The middle of the graft takes a diffuse eosin stain with only an occasional nucleus. Corresponds to the fascicular layer of the suprarenal cortex. A rim of cells on the convex border of the graft corresponding to the glomerular layer, and a thicker area of cells on the convex side of the graft, including in part cells that look like those of the suprarenal medulla, stain well. No evidence of connective tissue invasion of the graft or surrounding kidney.

**Case IX.** — July 18, 1903. Male Belgian hare. Morphine and ether narcosis. Operation per abdominal route. A little more than half of the left suprarenal introduced into the cortex of the left kidney. No suprarenal stump left. Kidney wound closed with two silk sutures. Abdominal wound closed with one row of silk sutures and one of chromicized gut. Nine days later rabbit found in cage with abdominal wound torn open and intestines exposed. Killed with ether. Graft found well imbedded in left kidney. No evidence of infection. Hardened in alcohol. Imbedded in collodion, sections cut, and stained with hemotoxin and eosin.

*Microscopic examination.* — Larger portion of graft necrotic. Fairly large portion (about  $\frac{1}{4}$  of graft) which stains well and is apparently alive. This consists principally of cells, resembling in structure and arrangement those of the suprarenal medulla. Some of the cortical portion of the graft has also survived. Fragmentation of nuclei is particularly well marked in the periphery of graft. Blood vessels at periphery dilated and filled with blood. Considerable evidence of parenchymous inflammation in surrounding kidney. Stage of productive inflammation not reached.

**Case X.** — July 22, 1903. Female Belgian hare. Hyperdermic of morphine sulphate, 3 cgm.; ether anesthesia. Abdominal route. Left suprarenal divided into halves by a median longitudinal incision. One-half introduced into cortex of left kidney near lower border and secured by two silk sutures. Abdominal wound closed as in previous operations. Rabbit made an uneventful recovery.

October 16, 1903, the right suprarenal was removed entire through the dorsal route. Right suprarenal did not appear to be hypertrophied.

Rabbit made a complete recovery from this operation and was apparently normal in all respects up to November 6, 1903, when it was killed for the purpose of examining into the state of the graft.

*Autopsy.* — Both abdominal and dorsal wound perfectly healed. All the abdominal and thoracic viscera appear normal. After most careful search by ourselves and others, no accessory suprarenal could be found, and no stump of the former suprarenals which had been removed. Right kidney appears normal. Left kidney shows a linear scar about 12 mm. long near lower border, but no other trace of graft externally. Kidney tissue,

including scar, excised and placed in Zencker's fluid. Animal was well nourished with plenty of subcutaneous and subperitoneal fat. No evidence of fat necrosis or other areas of necrosis anywhere. Urine shows no sugar or albumen. No pigmentation of mucosæ.

*Microscopic examination.* — Sections stained with hemotoxilin and eosin and also with Borel's stain to bring out connective tissue. Remains of graft show as one large mass of cells and several smaller islands, separated from the main mass and the kidney by new-formed connective tissue. The main mass and islands are separated from the surrounding kidney substance by a thick layer of connective tissue which also penetrates to some depth into the kidney cortex in the form of a wedge. This probably has replaced a large part of the graft which has disappeared. Greatest length of graft is 4 mm. ; greatest width is 2 mm. ; estimated thickness is 0.8 mm.

The cells of the graft are all of the same type, large, irregularly polyhedral to spherical, arranged in short anastomosing chains, separated by clear spaces. The nuclei stain well, the cytoplasm faintly. Many of the cell-bodies contain yellowish brown granular pigment. They are apparently of the type of the cells forming the medulla of the normal gland. No nerve cells or fibres were demonstrated. New-formed blood vessels found between the connective tissue strands and among the cells of the graft itself. Kidney immediately surrounding graft shows evidence of productive inflammation. Silk sutures had penetrated periphery of graft, but with very little evidence of inflammatory reaction.

**Case XI.** — September 15, 1903. Male Belgian hare. Left suprarenal removed entire and  $\frac{3}{8}$  of gland transplanted into cortex of left kidney. Surface of graft flush with kidney surface. Rabbit recovered from operation. November 18, 1903, in attempting to remove the right suprarenal via the dorsal route, the animal died suddenly from the anesthetic.

*Autopsy.* — Graft shows as yellowish mass about 3 mm. square, protruding from surface of left kidney. Hardened in Zencker's fluid. Imbedded in collodion. Sections cut and stained with hemotoxilin and eosin. Microscopic examination shows a few small, scattered groups of living cells, which are difficult to classify. The major portion of the graft is necrotic.

**Case XVIII.** — November 25, 1903. Rabbit. One-half of left suprarenal transplanted into cortex of left kidney. April 25, 1904, remaining right suprarenal removed. Rabbit killed June 1, 1904. At autopsy, abdominal viscera normal. Mesentery and small intestine slightly adherent to left kidney, at site of graft. In location of former left suprarenal, a body the size of a grain of rice was found, resembling in appearance suprarenal substance. No stump at site of former right suprarenal. Graft almost entirely absorbed. A small nodule remains well imbedded in

kidney. Microscopically the graft consists of cells of the type of those found in the zona reticularis and the medulla of the normal gland. The peripheral cells stain poorly, the central cells stain better. These consist of irregularly anastomosing columns separated by clear spaces. Nuclei stain clearly with hemotoxilin. Cytoplasm contains fine brownish pigment. Graft walled off from kidney by connective tissue. Numerous large blood vessels lead up to graft, but do not penetrate it. Suprarenal stump consists of medullary cells surrounded by a thin band of connective tissue, containing numerous blood vessels filled with blood which penetrate into gland.

**Case XIX.**—December 11, 1903. Belgian hare. Middle third of left suprarenal transplanted into lower border of cortex of left kidney. Surface of graft exposed. August 22, 1904, the remaining right suprarenal was removed. Animal made a good recovery. Killed with ether, September 12, 1904. At autopsy, found abdominal organs normal. No stump of either suprarenal remained. Found a small spherical nodule on renal vein midway between kidney and inferior vena cava. This, upon microscopical examination, was found to be an accessory suprarenal.

Microscopical examination of the graft shows it as a small nodule, separated from the kidney and surrounded by a thin band of connective tissue with several smaller islands under the kidney capsule. The larger nodule consists of cells that stain well and which have an arrangement similar to that of the zona fasciculata of the normal gland. The smaller islands consist of fine granular cells that stain well and have the appearance and arrangement of the suprarenal medulla. These cells also take a greenish hue with ferric salts, and a brown tinge with chrome salts. Fine connective tissue stroma in the larger nodule consisting of thin fasciculæ separating the columns of cells.

**Case XX.**—January 14, 1904. Belgian hare. Middle third of left suprarenal inserted into incision in lower border of cortex of left kidney. Graft not completely buried. Somewhat folded. July 21, 1904, the remaining right suprarenal was removed. Apparently slightly hypertrophied. Considerable hemorrhage. In middle of operation artificial respiration had to be employed. Died two hours later.

At autopsy, found abdominal viscera normal. No suprarenal stumps or accessory glands. Graft found in scar of kidney as a round yellowish spot, 1 mm. in diameter. Microscopical examination shows one large mass of well-staining cells, arranged in parallel columns with very little connective tissue between, and surrounded by a thick band of connective tissue. Several smaller groups of cells in the scar of the wound are typical medullary cells with brown pigmented cytoplasm. The tissue was hardened in Zencker's fluid.



**Case XXI.**—July 29, 1904. Belgian hare. Middle third of left suprarenal transplanted into left kidney. Graft not completely buried. November 10, 1904, right suprarenal removed. Gland appears to be hypertrophied. November 11, rabbit appears to have recovered from operation, but head is slightly twisted to left side. This becomes more marked during day. When disturbed moves in circles toward left and falls to left side. Left pupil does not react to light, but remains contracted. Right pupil dilated, but contracts to strong light. Tail twists toward the left. This condition persisted, gradually growing more marked until November 21, 1904, when the rabbit was killed.

At autopsy, found a small, partly organized blood clot over left cerebellar hemisphere. Abdominal viscera normal. Cheesy abscess behind right kidney. Large cheesy mass connected with wound of left kidney. On section the remains of graft were found intact below this mass. No accessory suprarenals or suprarenal stumps were found. Left kidney and graft hardened in Zencker's fluid. Microscopical examination shows graft measuring 3 by 4 mm. consisting of a thin zone of cells arranged like those of the zona reticularis, and thicker masses of cells with distinct nuclei and poorly staining cytoplasm arranged like those of the suprarenal medulla. The graft is walled off from the kidney by a thick layer of connective tissue containing blood vessels which penetrate the graft.

**Case XXII.**—August 1, 1904. Mixed Belgian hare. One-third of left suprarenal transplanted into left kidney. Technical result good. September 30, 1904, removed right suprarenal. Animal made a good recovery and was apparently normal until the evening of October 28. Found dead in cage October 29, in position of opisthotonos.

At autopsy everything appeared normal with the exception of a number of cysts attached to mesentery and under surface of liver. Graft well imbedded in cortex of left kidney, 4 mm. wide, 6 mm. long, and 1.5 mm. thick. Upon microscopical examination, found to consist of cells whose nuclei stain well with hemotoxin, and having a large cell body which stains but faintly. These cells belong in part to the medullary layer, and in part to the reticular layer of the suprarenal cortex. The graft is penetrated by a network of fine connective tissue fibres.

**Case XXIV.**—August 26, 1904. Mixed Belgian hare. One third of left suprarenal introduced into cortex of left kidney. October 6, 1904, right suprarenal removed. Animal killed October 25, 1904.

*Autopsy.*—Rabbit rather poorly nourished. Intestines and stomach empty. No accessory adrenals or adrenal stumps remaining. Graft well imbedded in left kidney. Size of graft,  $10 \times 6 \times 7$  mm. Hardened in Zencker's fluid. Microscopically consists of large cells with nuclei that stain well and cytoplasm that stains faintly, apparently of medullary type. Fine connective tissue stroma and numerous new-formed blood vessels.



- Case XXV.**— December 6, 1904. Domestic rabbit. One-third of left suprarenal transplanted into left kidney. January 6, 1904, remaining right suprarenal removed. Considerable hemorrhage and shock. During night temperature of room fell almost to freezing. Animal found dead in cage the following morning, fourteen hours after operation. Graft found well imbedded in cortex of left kidney. Major portion extracted with salt solution and injected into vein of another rabbit. Experiment spoiled because of clot in arterial cannula. Remainder of graft hardened in Zencker for microscopical examination. Cells of graft stain well. Belong to zona fasciculata, reticularis and medulla of normal gland.
- Case XXVI.**— April 15, 1905. Belgian hare. Middle third of left suprarenal transplanted into left kidney. During night of May 5, animal died. Cause of death undetermined. Graft found well imbedded. Removed crushed, and extracted with normal salt solution. Injection into vein of another rabbit gave no blood-pressure raising effect. Graft apparently inactive and probably necrotic.
- Case XXVII.**— April 21, 1905. Rabbit. One-third of left suprarenal transplanted to left kidney. Right suprarenal removed May 24, 1905. Rabbit died twenty-nine hours later with symptoms of suprarenal insufficiency. Graft extracted and injected into vein of another rabbit. No effect on blood pressure.
- Case XXVIII.**— April 27, 1905. Rabbit. Transplantation of one-third of left suprarenal into left kidney. Remaining right suprarenal removed June 1, 1905. Rabbit found dead, 8 A.M., June 3. Lungs congested. Areas of broncho-pneumonia. Pleural adhesions. Graft well imbedded, but rather too yellow to look normal. Extracted and injected into vein of another rabbit. Following injection, marked slowing of heart beat and slight fall in blood pressure. This was followed by acceleration of heart beat and rise of blood pressure, slightly above the normal. Not sufficiently positive to draw definite conclusions.
- Case XXIX.**— June 2, 1905. Female rabbit. One-third left suprarenal to left kidney. Right suprarenal removed September 9, 1905. Somewhat hypertrophied. Animal in poor condition of nutrition. Died following day, about fifteen hours after operation. Found lower half of belly filled with encapsulated cheesy mass (double psoas abscess). This was not connected with the suprarenal graft, which was found well imbedded in left kidney. Left kidney atrophied to two-thirds the size of the right kidney. Graft of good size and normal appearance. Hardened in Zencker. Microscopical appearance. Cells of graft stain well. Arrangement similar to that of zona reticularis and medulla of normal gland. Considerable connective tissue network throughout graft.
- Case XXX.**— June 3, 1905. Mixed Belgian hare. After removal of left suprarenal entire, one-third was transplanted into the cortex of the

left kidney. September 13, 1905, the remaining right suprarenal was removed. There was no apparent hypertrophy of this gland. Rabbit was found to be pregnant. Made a good recovery and had litter of young, October 7, 1905. Continued in good condition until November 14, 1905, when the left kidney, containing the suprarenal graft, was removed. Recovered from the operation and seemed to be in good condition until November 17, 1905, three days after the removal of the graft. Now appeared sick, remained huddled up in a corner of the cage. When disturbed, would raise itself upon its haunches but immediately drop back to sitting posture. Efforts at movement followed by tremor of muscles. Weakness progressively increased during day. Died night of November 17. No convulsions.

*Autopsy.*—Rigor mortis firm. No evidence of death in convulsion. Fairly well nourished. Considerable gas in small intestine. Intestinal vessels injected with blood. Bladder contains 2 c.c. straw-colored fluid. Right kidney appears normal. No accessory suprarenals or other suprarenal remains could be found. No unusual pigmentation of mucous membranes. All organs appear normal. Pieces preserved for microscopical examination. Both ventricles of heart dilated. Urine shows trace of albumen; no sugar; no casts.

Graft shows in left kidney as a yellowish white oval mass, a large part of the surface of which is flush with the surface of the kidney. Surface area in longest and widest part is  $9 \times 7$  mm. About 4 mm. is imbedded in cortex. Part of graft and kidney transferred to Zencker for microscopical examination. Larger part of kidney with graft preserved in kaiserling.

Microscopical examination of graft shows it to consist of cells, mostly with small nuclei and large amount of cytoplasm, part of which has a brownish granular appearance. The structure, in the main, resembles that of the zona reticularis and suprarenal medulla. Fine connective tissue stroma throughout graft. Numerous blood vessels in connective tissue about graft and penetrating graft itself.

**Case XXXI.**—June 7, 1905. Large Belgian hare. Removed left suprarenal and transplanted middle third to cortex of left kidney. October 9, in attempting to remove the right suprarenal, the inferior vena cava was injured and the animal died from hemorrhage and shock.

Graft found well imbedded in left kidney as a wedge-shaped piece 2 mm. deep by 2 mm. wide by 3 mm. long. Appears rather too yellow to give promise of survival. Small piece hardened in formalin for sectioning. The rest was extracted for possible blood-pressure raising principle. Extract does not reduce Fehling's solution. Both vagus nerves of a rabbit were cut, and then the graft extract was injected into jugular vein. Several seconds later, a transient small rise of blood pressure occurred,

followed by a fall and a second rise. These rises in blood pressure were not due to struggles on the part of the animal, but were also not sufficiently well marked to be ascribed to the presence in the extract of a blood-pressure raising principle.

Microscopical examination of graft shows a few cells apparently alive, but the major part of the graft is necrotic.

#### SUMMARY OF RESULTS.

Of the thirty-two experiments of suprarenal grafting in the same animal, recorded in part above, one case (XXX) satisfies all the conditions of complete proof of the functional survival of the graft. One hundred and two days after the removal of the left suprarenal and introduction of the graft into the kidney, the right suprarenal was removed. Sixty-two days later, the kidney containing the graft was removed. About three and one-half days after the removal of the graft the animal died with symptoms of suprarenal insufficiency. At autopsy no trace of suprarenal tissue could be found. The graft was found to consist of living suprarenal cells, including those of the medulla. The conclusion is therefore justified, we believe, that the animal was kept alive for sixty-two days by the suprarenal graft alone, in the absence of other suprarenal tissue. The total time from the introduction of the graft to its removal was one hundred and sixty-four days.

Partial proof of the functional survival of suprarenal grafts was obtained in nine other cases. Of these, in the light of Case XXX, we believe that we are justified in inferring that the graft alone was responsible for the continued life of the animal where other suprarenal tissue was absent, in Cases X, XXI, XXII, XXIV.

In Case XVIII the animal survived the second operation, and the graft was found histologically of the same appearance as those mentioned above, but there was also found a stump remaining at the site of the left suprarenal which was composed of medullary cells. The graft was probably functioning and would have been sufficient to preserve the suprarenal function in the absence of the stump.

In Case XIX the same statement may be made that was made for Case XVIII, only, in this case, an accessory suprarenal body was found.

In Cases I, XX, and XXIX, the grafts were found to contain suprarenal medulla, but no further proof of function was obtained for the

reason that they either did not come to the second operation or died through accident at this time.

Proof through demonstration of blood-pressure raising principle, still remains to be obtained. Where it was attempted, the results were negative. In these cases where histologic examination was likewise made the grafts were shown to have undergone necrosis, In the other cases it was felt that there was not sufficient material for extraction and for histologic examination, and the latter seemed to be the more important.

Living grafts were found the following lengths of time after introduction into the kidney cortex: 188 days; 247 days; 189 days; 61 days; 105 days; 60 days; 99 days; 164 days; 108 days. It may be questioned whether these grafts would have continued to survive or would have eventually been replaced by connective tissue. It would seem, however, from the histologic appearances of the grafts and the presence of new-formed blood vessels that the process of atrophy and connective tissue encroachment had reached its limit. Furthermore, Stilling has shown survival of suprarenal tissue more than three years after transplantation.

In conclusion, it is believed that this series of experiments has clearly demonstrated the possibility of suprarenal transplantation with preservation of function. The uniform success of this operation will depend upon perfection of technic. Whether or not transplantation of the suprarenal from one animal to another of the same species or of closely allied species, or from one species to another, will meet with success, remains to be demonstrated. Other series of experiments with this object in view are now under way.

## EXPLANATION OF FIGURES.

FIGURE 1. — Whole section, Case XXIV, enlarged 10 diameters. Stain, hemotoxin and Mallory's stain for connective tissue. The transplanted suprarenal shows in the upper part of the figure. Enlargement, about 10 diameters.

FIGURE 2. — Whole section, Case XXX. Stain, hemotoxin and eosin. The transplanted suprarenal shows in the upper part of the figure. Enlargement, about 10 diameters.

FIGURE 3. — Photomicrograph of section through kidney and graft of Case X. Cells of suprarenal show in right of figure. Left of figure shows connective tissue which separates the graft from the kidney. Magnification, about 400 diameters.

FIGURE 4. — Photomicrograph of section through kidney and graft in Case XXX. Suprarenal graft shows in right of figure. Magnification, about 400 diameters.



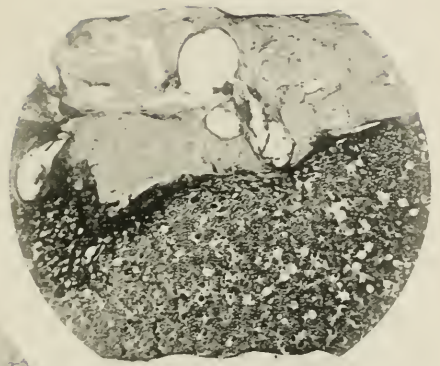


FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.





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A REFLEX RESPIRATORY CENTRE.

By IDA H. HYDE.

I SHOWED that in *Limulus* each ganglion of the ventral nerve cord is a centre for respiratory movements, and can be isolated from the other parts of the nervous system, or sectioned into two lateral halves without interrupting the respiratory movements of the particular segment, or of the lateral half to which it belongs.

Consequently we possess in *Limulus* most favorable conditions for the investigation of the automatic or reflex character of the respiratory centre. The proposition resolves itself into noting the effect when all possible afferent paths are eliminated without injury to the ganglion and motor fibres.

I found that the four main branches of the afferent anterior root, coming from the heart, intestine, longitudinal muscles, and carapace, contain both augmentor and inhibitory respiratory fibres, and can be cut with cocaine or by freezing without stimulating or interrupting the respiratory activity.

The difficulty of paralyzing the sensory and not the motor fibres in the posterior root, which innervate the gills, was finally overcome either by stimulating with strong induction currents for about 35 seconds, or employing from 0.2 to 0.5 per cent cocaine hydrochloric acid from 10 to 30 minutes. It requires in all cases more prolonged action to paralyze the motor fibres.

When all possible afferent paths to the centre have been cut, and the motor paths from the centre are still intact, the respiratory movements at once cease. And when the effects on the sensory fibres of either the cocaine or electrical stimulation have worn off, the respiratory movements return again, thus proving that the respiratory centres in *Limulus* are reflex in character.

ON THE RELATION OF THE COAGULATION OF THE COLLOIDS OF THE CTENOPHORE SWIMMING-PLATE TO ITS CONTRACTILITY.

BY R. S. LILLIE.

THE normal rhythm of the swimming-plate of *Eucharis lobata* is usually from three to six beats per second. No change is seen in the substance of the plate during normal contraction. In certain solutions (*e. g.*, alkaline  $\frac{6}{10}$  m. sodium chloride) the rhythm is greatly accelerated, and individual beats become indistinguishable. This abnormally active movement is always accompanied by a progressive coagulation of the colloids of the plate, the originally clear substance of which becomes clouded and finally opaque, usually within half a minute or less; movement then ceases. The coagulation does not begin until the plate has begun vibrating; inactive plates remain transparent.

A connection between a coagulation of the colloids of the plate and its contractility is thus indicated. Probably the contraction-phase in the normal beat is due to a partial coagulation of the colloids; this change is reversed during the relaxation phase. The above solutions accelerate the coagulative phase of the process; hence the accelerated movement and the progressive coagulation of the colloids.

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CONCERNING PEPTONE (SECOND COMMUNICATION).

BY L. B. STOOKEY.

THIS paper is a continuation of a study of peptone carried out in the laboratory of Professor Hofmeister. One of the fractions has been studied further—the one designated as “I B Benzoyl Chloride.” This fraction gave the biuret, Molisch, and extremely faint xanthoproteic reactions. The Hopkins and Millon tests were negative. Sulphur was not present. Ten grams were boiled with 5 per cent sulphuric acid until the biuret reaction disappeared. A residue remained. This residue gave the Molisch reaction more intensely than the original substance. It is not impossible that the residue was a glucosamin benzoyl chloride compound. The filtrate was examined in the usual manner. Neither arginin nor histidin could be detected. Lysin was present and was identified as the picrate.

Neither glutamic nor aspartic acid could be found. Alanin was isolated and identified as a copper salt.

A further study of this fraction is in progress. On account of the small quantity of substance examined these findings cannot be looked upon as conclusive, yet the fact that a condensation product of 2 benzoyl groups, 1 lysin, 1 glucosamin, and 1 alanin, would have the composition of: C 57.80 per cent, N 9.30 per cent, H 6.31 per cent, O 26.57 per cent, while this fraction gives as follows: C 58.68 per cent, N 8.96 per cent, H 5.88 per cent, O 26.48 per cent, may be regarded as suggestive, indicating a possible molecular formula of  $C_{29}H_{38}N_4O_{10}$ .

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#### THE "ANTITOXIC" INFLUENCE OF CERTAIN ANIONS ON THE ACTION OF SOLUTIONS OF ALKALINE EARTH CHLORIDES ON CILIATED CELLS.

By R. S. LILLIE.

THE destructive action of solutions of calcium chloride, barium chloride, and strontium chloride on the ciliated cells of *Mytilus* is apparently due to the preponderant action of the cation. Addition of salts with strongly acting anions was found markedly to retard this action. Thus in  $\frac{m}{2}$  strontium chloride the gill filaments undergo gradual coagulation; this action is prevented and movement is prolonged from a few minutes (duration in pure strontium chloride) to periods ranging from ten to sixty hours by addition of small quantities of sodium hydrate, potassium cyanide, sodium bromate, sodium hydrogen arsenate. Other anions (Br, I, CNS) proved also effective, though to a less degree. On the other hand, toxicity due to a preponderance of the anion (as in sodium salts) is counteracted by cations.

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#### THE ALIMENTARY ENZYMES OF THE EMBRYO.

By LAFAYETTE B. MENDEL.

THE observations on the occurrence of enzymes in the alimentary tract of the embryo, here briefly summarized, form part of a study of the development of physiological processes in growth and the

equipment of growing organisms for their nutrition. With the cooperation of Mr. P. H. Mitchell, extracts of the entire intestine of embryo pigs of various ages have been examined by a diversity of methods for sucrase, maltase, and lactase. Maltase appears to be the most universal of all these enzymes. In the embryo pig lactase is present very early, while sucrase cannot be detected, even in very large embryos. All control trials with extracts of other embryo tissues, such as kidney or liver, gave negative results. The extracts prepared from the intestines of adult pigs contained no lactase, but always afforded sucrase reactions. Suckling pigs yielded all of the enzymes mentioned. In the dog there likewise appears to be a difference between the early and adult stages as regards their alimentary enzymes. Lactase was not found in either the newly hatched chick or the adult hen, while sucrase was obtained from the intestine of each. The search for pepsin and rennin in the embryonic stomach yielded entirely negative results even in eleven-inch pig embryos, thus agreeing with the observations of the majority of earlier investigators. The relation of these specific occurrences of the sugar-inverting enzymes to functional adaptations, such as the newer experiments of Weinland and of Bainbridge have suggested for the animal body, deserves further study.

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#### THE RELATION OF CARDIAC INHIBITION TO THE INORGANIC CONSTITUENTS OF THE BLOOD.

By W. H. HOWELL.

THE heart of the terrapin and the frog were kept beating on an artificial circulation of Ringer's fluid, the inflow cannula from the reservoir being placed in the left superior vena cava or inferior vena cava, and the outflow cannula being inserted through the semilunar valves so as to open within the cavity of the ventricle. The vagus was stimulated in the neck; in the case of the frog the combined vagus and sympathetic was stimulated. The sensitiveness of the heart to vagus inhibition was estimated in terms of the minimal strength of an induced current, expressed in graduations of the scale necessary to produce a perceptible slowing of the rate, or in some cases by the minimal stimulus necessary to produce complete cessation. The effects upon the ventricle and the auricles were studied separately.

In some cases the contractions of each chamber were recorded, while in others the heart was left entirely undisturbed.

*Increase in the potassium contents.*—The hearts were irrigated first with a standard Ringer's solution containing 0.03 per cent potassium chloride. Increase in the amount of potassium chloride to 0.05, 0.07 per cent, or higher, caused an increased sensitiveness to vagus inhibition. If, however, the potassium contents were increased to the point of causing marked potassium inhibition, the heart showed a very greatly diminished sensitiveness to vagus inhibition.

*Increase in calcium contents.*—These experiments were made only upon the heart of the terrapin. In this animal there is reason to believe that the vagus nerve does not affect the ventricle directly. Increase of the calcium contents diminished the sensitiveness of the ventricle to vagus inhibition, and at a certain concentration suspended the inhibitory action of the vagus entirely. At these latter concentrations the amount of calcium salts present (0.10 per cent) was sufficient to cause the ventricle to beat independently in spite of the complete inhibition of the auricles. So far as the auricles were concerned, increase of the calcium concentration had no effect on the vagus inhibition.

*Effect of entire removal of potassium salts from the circulating liquid.*—The results were somewhat variable, but in most cases the inhibition of both auricle and ventricle was very greatly reduced or absent altogether, suggesting the hypothesis that vagus inhibition is possible only when potassium salts in diffusible form are present in the tissue.

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## A STUDY OF THE METABOLISM OF THE NERVOUS SYSTEM.

By W. KOCH.

THE investigations of Ehrlich and of L. Hill indicate that the cerebellar cortex needs to be constantly supplied with an excess of easily available oxygen in order to carry on its function. In another investigation L. Hill gives results which indicate that this oxygen is not used to any extent for the production of carbon dioxide. In a number of other investigations attempts have been made to emphasize the importance of phosphoric acid as a product of nervous activity. As



phosphorus under normal conditions always enters the system in an oxidized form, it could not be derived from a metabolism involving oxidations, but would rather result from the destruction of phosphorized lipoids and nucleins. The low carbon dioxide formation speaks against Loew's hypothesis, that these lipoids are a source of energy to nerve tissues.

The view is here put forward that there is in the nervous system a metabolism by means of which there is elaborated from proteid a compound or compounds of fairly high molecular weight, which compounds no longer give proteid or peptone reactions, but resemble rather the polypeptids. The compounds so far isolated, contain up to four per cent of sulphur in a form easily split off as sulphates and no longer present as cystin sulphur. The change in the state of oxidation of the sulphur gives a clue as to the rôle played by oxygen in this metabolism. Evidence of the importance of these compounds to the nervous system is based on the following:

1. A comparison of the quantity of these compounds in nerve tissues with the amount found in other tissues.
2. An examination of cerebro-spinal fluid during life and post mortem.
3. A chemical analysis of the cortex in cases of dementia præcox.

The rate of this metabolism has not yet been determined, nor the particular part of the cortex where it takes place.

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## MOVEMENTS OF AMCÆBÆ AND ALLIED FORMS.

BY C. F. HODGE (FOR MR. O. P. DELLINGER).

THE key to the movements of most forms is found in the *Diffflugias*. The shell may be lifted off the substratum and carried (*D. spiralis*) or dragged on the surface (*D. acuminata*), but the method is the same. A long pseudopod is extended, attached by suction at its tip, and the body drawn up to the point of attachment. While the first pseudopod is shortening, its substance goes to form a new pseudopod, which is thrust out free in the water, and, when fully extended, is brought around to the line of advance and in turn attached at its tip, and the above cycle is repeated.

Most amcæbæ, *proteus*, *radiosa*, *limax*, and several others, move in precisely the same manner. Pseudopods are extended in the line of

advance, attached at their tips, and the body squeezed and pulled up to the point of attachment. These pseudopods may be many or few, long or short, and the whole amoeba may be extended and attached at the ends, and thus progress much after the fashion of a measuring worm. There is in these forms no rolling of the ectosarc, as described by Jennings. Particles attached to the surface retain their relative positions with reference to the rest of the body, much as would particles of dust on the back of a caterpillar. The rolling of the ectosarc is avoided by lunging from side to side, or, what amounts structurally to the same thing, by walking on slender pseudopods. Movements are characteristically different when travelling and when searching for food.

In *A. verrucosa*, the form upon which Jennings apparently did most of his work, the ectosarc rolls as he describes, but the normal side view is not at all like the figures he gives. This form, however, moves by alternating points of attachment at both anterior and posterior ends.

The apparatus used for obtaining side views of Rhizopods is simple, consisting of an ordinary thin slide having the edge ground and polished at right angles with the surface, and with long cover-slips cemented to both surfaces and extending beyond the polished edge. This leaves a narrow trough into which the animals are pipetted, and with the tube of the microscope brought to the horizontal position, they move along the edge of the slide.

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## A LAW OF GROWTH REITERATED.

By GRAHAM LUSK.

OPPENHEIMER<sup>1</sup> first called attention to the fact that the gain in weight of infants is directly proportional to the quantity (or calories) of milk ingested. Thus two different children during the second month of their lives gained 191.2 and 201.1 gm. for each kilogram of milk given; in the third month, 120.3 and 138.5 gm.; in the fourth month, 102.6 and 103.3 gm.

Dr. Margaret B. Wilson<sup>2</sup> showed that new-born suckling pigs of different weights gained 218, 215, 213, 222, and 213 gm. in weight

<sup>1</sup> OPPENHEIMER: *Zeitschrift für Biologie*, 1901, xlii, p. 147.

<sup>2</sup> WILSON, M. B.: *This journal*, 1902, viii, pp. 195-197.

per 1000 calories in the milk ingested. Rost<sup>1</sup> gave meat fat and bone ash to three dogs of the same litter, the experiment starting on the ninety-eighth day of their lives and continuing eighty-eight days. The dogs weighed at the start 3.2, 2.2, and 4.1 kilograms. The writer calculates their gain in weight per 1000 calories ingested as 122, 141, and 134 gm.

Dr. Wilson found in pigs that there was a retention for growth of 18 to 19 per cent of the calories of the food. In a child of seven weeks Camerer found 15 per cent; Rubner and Heubner in one of seven and a half months, 12.2 per cent so retained. This retention of a definite nutritive factor of the food is a necessary corollary to a growth which is proportional to the calorific intake.

It is noteworthy that the appetite determines the regular ingestion of sufficient energy for the life processes, plus a small but fixed extra percentage necessary for growth.

Strictly formulated, this law of growth is that in the development of the normal young of the same age and species a definite percentage of the energy content of the food is retained for growth irrespective of the size of the individual.

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## THE CAUSE AND PHENOMENA OF SURGICAL SHOCK.

BY YANDELL HENDERSON.

FROM fifty dogs the writer has recorded the volume changes of the ventricles (by means of a plethysmograph) simultaneously with the arterial or intraventricular pressure. Excepting a few cases in which extensive hæmorrhage occurred, or in which an excess of anæsthetic was administered, the course of events leading to the death of these animals was entirely different from that described by Crile. In several experiments the dog was kept under observation for six hours or more after the thoracic viscera had been exposed. The first sign of the approach of "shock" was an increased rapidity of the heart beat. Arterial pressure was at this time unaltered. As the heart beat became more and more rapid, the amplitude of the volume-curve of the ventricle (which affords a measure of the volume of the systolic discharge) was progressively diminished. During this stage systolic pressure in the aorta remained at its previous level; diastolic

<sup>1</sup> ROST: Arbeiten aus dem kaiserlichen Gesundheitsamte, 1901, xviii, p. 206.

pressure on the other hand rose considerably. The amplitude of the pulse or "pulse pressure" was therefore markedly diminished. Finally a stage was reached in which one heart beat followed another so rapidly that the diastolic interval was insufficient to allow the ventricles to fill. The discharge of blood into the arteries was therefore so reduced in amount that arterial pressure fell rapidly, and death occurred. It is generally held that "cardiac muscle cannot be tetanized"—although Danilewsky has recently called this statement in question. The writer believes that the changes in the heart above described cannot be properly characterized otherwise than as a "tetanus of the heart."

This progressively increasing rapidity in the heart beat culminating in tetanus is probably due to a diminished carbon-dioxide content of the blood.

Erlanger holds that the pulse pressure is proportional to the discharge-volume of the ventricle. With this view the data obtained in these experiments are in general accord. This is important, since it suggests a method for determining in the clinic whether the events leading to "shock" in the human subject are identical with those in the dog. If such is the case, an increasing pulse rate and a rise of diastolic pressure are the indications of the approach of "shock."

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## THE INFLUENCE OF ALUMINIUM IONS ON LUPIN SEEDLINGS.

BY H. D. HOUSE AND WILLIAM J. GIES.

ALTHOUGH aluminium compounds appear to exist in small proportions in many plants and occur in traces in some animals, relatively little has been learned regarding the biological effects and significance of aluminium. The authors have begun a series of studies in this connection, the first of which has been an investigation of the influence of aluminium on lupin seedlings. The methods described by True and Gies (1903) were used, and the following compounds were taken: aluminium sulphate, aluminium nitrate, aluminium chloride, aluminium-sodium chloride, potassium alum, and ammonium alum. The subjoined summary presents some of the more significant results, which represent the average of very many observations for periods of forty-eight to seventy-two hours.

In nearly all cases, in these experiments, little or no effect was produced at a concentration of  $\frac{m}{65536}$ . In concentrations greater than  $\frac{m}{65536}$ , growth usually was markedly inhibited. In concentrations less than  $\frac{m}{65536}$  and down to  $\frac{m}{1048576}$ , or  $\frac{m}{2097152}$ , growth was stimulated as a rule. The influence of electrolytic dissociation and other physical factors will be discussed later.

Aluminium compound.	Greatest molecular concentration that permitted slight initial growth.	Least molecular concentration in which there was no growth after 24 hrs.	Greatest molecular concentration in which growth was equal to that in the pure water control.	Molecular concentration in which initial stimulation took place.	Molecular concentration in which the maximum stimulation occurred.	Least molecular concentration in which growth was again equal to that in the pure water control.
$Al_2(SO_4)_3$ . .	$\frac{m}{x}$ 8,192	$\frac{m}{x}$ 16,384	$\frac{m}{x}$ 65,536	$\frac{m}{x}$ 131,072	$\frac{m}{x}$ 1,048,576	$\frac{m}{x}$ 4,194,304
$Al(NO_3)_3$ . .	1,024	16,384	65,536	131,072	1,048,576	2,097,152
$AlCl_3$ . . .	2,048	2,048	65,536 *	131,072	131,072	2,097,152
$AlCl_3, NaCl$	16,384	32,768	65,536 *	131,072	131,072	2,097,152
$KAl(SO_4)_2$ .	1,024	4,096	32,768	65,536	{ 131,072 } { 524,288 }	2,097,152
$NH_4Al(SO_4)_2$	8,192	16,384	131,072	262,144	524,288	4,194,304

\* About midway between this concentration and  $\frac{m}{131072}$ .

## THE MECHANISM OF SALT GLYCOSURIA.

BY FRANK P. UNDERHILL AND OLIVER E. CLOSSON.

WHEN sodium chloride is injected into the venous circulation of the rabbit, polyuria and glycosuria are in evidence, probably as a result of an increased permeability of the kidney. The permeability of the kidney may be decreased by injection of a mixture of sodium chloride and calcium chloride, as is indicated by the temporarily decreased flow of urine and diminished or inhibited excretion of sugar. Further evidence that this form of glycosuria is of renal origin is furnished by the observation that during the appearance of sugar in the urine hypoglycæmia is noted, whereas the sugar content of the blood becomes normal, or hyperglycæmia obtains, when the excretion



of sugar in the urine has been inhibited by injections of a mixture of sodium chloride and calcium chloride. Injections of sodium chloride into the cerebral arterial circulation produce glycosuria with no polyuria but with an accompanying hyperglycæmia. The increased sugar content of the blood may be referred to disturbances of respiratory processes, dyspnœa, induced by injection of sodium chloride. The mechanism controlling the glycosuria provoked by the introduction of sodium chloride into the circulation of the rabbit, therefore, varies with the manner of injection. Injections of magnesium sulphate into the ear vein of the rabbit cause the appearance of sugar in the urine without an accompanying polyuria. The mechanism involved may be attributed to the dyspnœa induced.

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#### SOME RESULTS OF CENTRIFUGALIZING THE EGGS OF ARBACIA.

By E. P. LYON.

CENTRIFUGALIZING unfertilized eggs in the hæmatocrit for three to five minutes (10,000 revolutions a minute) effects a separation of the egg material into four distinct layers as follows: 1. A gray or grayish-yellow compact cap occupying a small area at the pole of least specific gravity. 2. A perfectly hyaline layer extending from the cap nearly to the equator. The nucleus lies in this layer against the gray cap first mentioned. The hyaline layer is so clear that the spindles may later be seen in it very clearly. 3. A gray granular layer extending from a little above the equator over two-thirds of the lower hemisphere. The substance of this layer shows a great tendency to redistribute itself after centrifugalizing. 4. A layer containing all the pigment of the egg. This layer occupies about one-third of the lower hemisphere.

Eggs so treated may be fertilized and will develop to plutei normal in all respects save pigmentation. The first segmentation is usually in the direction of the axis which has been established by centrifugalizing. In this case division begins at the upper pole. More rarely the first segmentation is nearly equatorial, though the pigmented blastomere is somewhat smaller than the upper, non-pigmented one. In other cases the first segmentation occurs at an



angle between the equatorial and polar planes. None of the various cleavage forms has, as yet, been followed through in detail.

The gastrulæ and especially the plutei from the centrifugalized eggs showed every possible distribution of pigment. More often it was in the arms or around the mouth. But sometimes it was confined to the gut. Or all of it might be in one side of the pluteus, the other being colorless. The distribution of the other substances in the pluteus has not yet been determined. Further work is being done.

In the eggs of other animals similar separations of material could be effected. In all eggs the nucleus is less dense than most of the other constituents.

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### A COLLOIDAL COMPOUND OF STRYCHNINE, AND ITS PHARMACOLOGY.

BY ORVILLE HARRY BROWN.

FRESH egg albumin, hydrogen dioxide, and a solution of a strychnine salt were mixed and allowed to stand. Usually the solution, when first made, has an appearance of an ordinary dilute egg-albumin solution. But after two or three days it gradually gets slightly milky, and after a couple of weeks it is noticeably more viscid. At the end of a month the solution has the appearance of a gel. The vessel containing it may be inverted without dislodgment of the material. The substance has a milky greenish-yellow tinge. The quantity of the gel is equal to the quantity of the original solution.

In one or two experiments a white flocculent precipitate occurred in place of the gel.

The gel is soluble in water. Heating the solution causes it to become clearer. Small amounts of acid cause a precipitate, soluble in excess. The heavy metals cause a precipitate. The substance reacts to Millon's reagent and to the xanthoproteic test. It is very slightly alkaline to litmus and neutral to phenolphthalein or congo red. It has been found that the presence of albumin and fat will prevent reactions for strychnine. Sugar does not. The substance was digested with acids and enzymes, but the strychnine reactions were never obtained.

The physiological test was then made. Two and one-half cubic centimetres of the gel were injected through a large hypodermic needle into the lymph spaces of frogs. After about five hours typi-

cal strychnine tetanus developed, and the frogs died in about twenty-four hours. About an hour elapsed between the first signs of the strychnine effects and the tetanus. Two controls were made. In one case water was substituted for the albumin and in the other for the hydrogen dioxide, and the same amount of each was injected into frogs. Typical strychnine tetanus developed in from three to five minutes, and the frogs died in the course of four or five hours.

### ON THE COMPOSITION OF NASAL MUCOUS MEMBRANE.

BY BERT RUSSELL AND WILLIAM J. GIES.

THE following percentage data on general composition represent average results of analyses of tissue from many oxen:

Portion.	Water.	Solids.	Organic Matter.	Inorganic Matter.
Anterior . . . . .	76.69	23.31	22.34	0.97
Median . . . . .	78.68	21.34	20.34	1.00
Posterior . . . . .	79.61	20.39	19.38	1.01
Longitudinal sections selected at random . . . . .	77.64	22.36	21.49	0.87
Transverse sections selected at random . . . . .	77.74	22.26	21.46	0.80

The quantity of ether-soluble material is equal to about 8 per cent of the solid matter. Reducing substance was absent from the aqueous extracts. Neither proteolytic nor amylolytic enzymes have thus far been detected. Autolytic changes will be investigated.

Much of the proteid in the tissue dissolves readily in water and ordinary salt solutions. Successive extractions of the fresh tissue in water, 5 per cent sodium chloride and 0.5 per cent sodium carbonate yielded solutions from which the following quantities of pure proteid (in terms of percentage of fresh tissue) were precipitated: Water, 4 per cent; sodium chloride, 2 per cent; sodium carbonate, 0.5 per cent. A collagenous residue, amounting to 10.5 per cent, remained.

Conspicuous among the soluble proteids present in the extracts is an acid-precipitable material, equal to about 2 per cent of the fresh tissue. Its properties have not yet been distinguished in detail. It appears to be nucleoproteid or a mixture containing nucleoproteid in

large proportion. It does not appear to be coagulable. Preliminary tests have failed to show the presence of mucoïd in the extracts.

Nearly 10 per cent of the fresh tissue is indigestible in artificial pancreatic juice, and gelatin is readily obtained from this residue. Only about 1 per cent of the fresh tissue remains undissolved in artificial gastric juice. This residue contains nucleïn.

The relations between composition and secretion will be considered at the conclusion of the study.

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### THE COMPARATIVE TOXICITY FOR PARAMÆCIA OF THE SALTS OF STRYCHNINE, OF MORPHINE, AND OF QUININE.

[By ORVILLE HARRY BROWN.

THE salts of the alkaloid were dissolved in distilled water in fractional normal concentrations. Solutions of equal concentration contain the same amount of pure alkaloid. Paramœcia were selected for testing the toxicity of the various solutions. The small amount of salt in one drop of culture water, in which the salt content is very low, allows practically no chance for chemical reaction between the alkaloid and the salt of the water. To two and one-half cubic centimetres of each solution of equal concentration were added two or three drops of water, containing hundreds of paramœcia. The length of time they continued to manifest evidence of life was noted and recorded. The experiments were performed under identical conditions of concentration, temperature, etc., for each solution. The death of the animals is evidenced by such decisive changes in protoplasmic structure, especially with some of the quinine salts, that there is no doubt regarding the end point. The time when the paramœcia cease to move was taken in each case as an indication of the complete toxic action of the solution used. An example selected from the protocols follows: the solutions are all of  $\frac{m}{100}$  concentration. In strychnine acetate the paramœcia lived thirty seconds; in the arsenate, fifteen minutes; in the bromide, eighty seconds; in the citrate, fifteen minutes; in the chloride, eighty seconds; in the glycerophosphate eight minutes; in the nitrate, two minutes.

Similar results were obtained with both morphine and quinine.

FURTHER OBSERVATIONS ON THE MECHANISM OF THE  
PYLORUS.

BY W. B. CANNON.

THE theory presented at the meeting of the Physiological Society in 1903,<sup>1</sup> that free acid in the stomach is the signal for the opening of the pylorus has received confirmation in feeding experiments and in observations through a gastric fistula. Various carbohydrate foods moistened with one per cent sodium bicarbonate, and various proteids fed as acid proteids have been observed in their exit from the stomach by means of the x-rays. The carbohydrates pass out much more slowly than when moistened with water, and the proteids pass out much more rapidly than the natural proteids. Observation through a gastric fistula placed just before the pylorus revealed that there was a concomitant variation of the first appearance of free acid in carbohydrate food in this region and the first discharge of this food from the stomach.

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THE MOVEMENTS OF THE STOMACH AND INTESTINE IN  
SOME SURGICAL CONDITIONS.

BY W. B. CANNON AND F. T. MURPHY.

FLUOROSCOPIC observations were made on animals etherized usually one half-hour, operated upon, and subsequently fed food mixed with a small amount of subnitrate of bismuth.

After high intestinal section and suture, gastric peristalsis runs quite normally, but for almost six hours after recovery from the ether the pylorus remains tightly closed against the peristaltic pressure and does not permit the food to enter the injured gut. The delay of the discharge from the stomach coincides remarkably with the period of primary cementing of intestinal wounds.

In case of intestinal obstruction food leaves the stomach without delay. As it accumulates above the obstruction, violent peristalsis repeatedly occurs, tending to force the food past the obstacle. The peristalsis alternates with vigorous segmenting movements. After such turbulent treatment for some time, the food has been seen

<sup>1</sup> CANNON: *This Journal*, 1904, x, p. xvii.

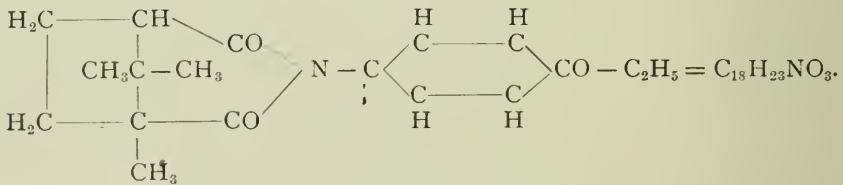
moving swiftly *backward* to the stomach along the course previously traversed from the stomach to the obstruction.

In studying the conditions of operation as possible causes of post-operative paralysis of the alimentary canal, etherization, one half or one and a half hours, was found not to delay to any marked degree the discharge of food from the stomach; exposure to the air likewise caused no noteworthy delay; unusual cooling of the gut resulted in some postponement of the initial passage of the food into the intestine; but by far the most striking effects were seen after handling the digestive organs. Even with most gentle handling, within the peritoneal cavity or under warm salt solution, no gastric peristalsis was seen and no food left the stomach for three hours. Fingering gently in the air usually caused still greater retardation of the movement of the food. And with rough handling in air no food passed from the stomach for four hours, and then it emerged very slowly and was moved through the small intestine with extreme sluggishness.

A STUDY OF PARA-AETH-OXY-PHENY-CAMPHORYL-IMID  
(CAMPHENAL) AS AN ANTIPYRETIC.

By E. M. HOUGHTON.

THIS substance is a condensation product having the following formula, as isolated by Heitmann and Clemensen:



Para-aeth-oxy-pheny-camphoryl-imid appears in the form of silky, colorless needles, odorless, tasteless, and permanent in the air. It is sparingly soluble in cold water, more soluble in hot water, and very soluble in organic solvents as alcohol, acetone, etc. The chemical formula for this substance differs from that of acetphenetidin in that an acetyl group has been replaced by a camphoryl group. It was believed that a chemical substance possessing this formula would per-



haps have the same antipyretic effect as acetphenetidin without the depressing effect upon the heart and circulation, since pharmacologists look upon camphoric acid and its relatives as possessing a direct stimulating action on the heart and central nervous system and these drugs are used in collapse. Several series of animal experiments were undertaken to demonstrate this property of the drug:

Series 1 shows that when administered internally to normal dogs in large quantities it produces practically no influence upon the temperature, while acetphenetidin shows an irregular but usually a considerable antipyretic action.

Series 2. A number of dogs suffering from "Dog distemper," whose temperature was abnormally high, were treated with camphenal without showing any marked antipyretic effect.

Series 3. Several dogs were given diphtheria toxin which possesses the property of producing an increased temperature. Camphenal in each case produced slight fall if any in temperature, but much less marked than in the case of acetphenetidin or acetanilid.

We would conclude from these experiments that the drug is not of value as an antipyretic, and in fact the experiments do not demonstrate that it would be of any value as a therapeutic agent. It is of interest, however, in showing that the camphoryl group cannot take the place of the acetyl group in acetphenetidin without the loss of the antipyretic property.

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#### FURTHER OBSERVATIONS ON THE ACTION OF LIPASE.

By A. S. LOEVENHART.

Using clear liver extracts of the beef liver, Magnus<sup>1</sup> found that its action on amyl salicylate disappears after dialysing for four days. The activity returned on adding to the dialysed extract a portion of liver extract which had not been dialysed, but which had been deprived of lipolytic activity by boiling. The action of liver extract on amyl salicylate depends, therefore, on two substances, one of which is destroyed by boiling and is not dialysable, — the enzyme, — while the other is not destroyed by boiling and is dialysable. This latter substance Magnus regarded as a coferment. I have found that the coferment is the bile salts. Dialysis does not cause the extract to lose

<sup>1</sup> MAGNUS: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 149.



its action on ethyl butyrate. Dry powders have been prepared from the pancreas and liver of the beef, dog, and pig, all of which possess markedly the power of hydrolysing the esters. Fresh extracts of the liver of the pig and suspensions of the pig liver powder decompose ethyl butyrate about three times as rapidly as the corresponding pancreas preparations; whereas its action on olive oil is only about one seventh that of the pig's pancreas. On heating dry pancreas powder at  $110^{\circ}$  the activity on ethyl butyrate suffers more than the activity on olive oil. Sodium fluoride inhibits the action of pancreas on ethyl butyrate more than on olive oil. On mixing extract of the pancreas with that of the liver, spleen, etc., the action on olive oil is accelerated often as much as 200 per cent; whereas, with ethyl butyrate, no acceleration occurs. These observations would indicate that the enzyme which acts on the higher fats, and which is properly called lipase, is probably different from that which acts on the esters. Whether these facts necessitate ascribing the hydrolysis of esters to a separate enzyme — esterase — will be fully considered in a subsequent paper.

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#### OBSERVATIONS ON THE SURVIVAL-RESPIRATION OF CURARIZED AND NON-CURARIZED MUSCLE.

BY G. T. KEMP AND E. R. HAYHURST.

THE results given below were all obtained from muscles supplied with abundant oxygen (five to eight litres per hour of air free from carbon dioxide) at a negative pressure of 25 mm. mercury.

1. Isolated frog and mammalian muscle, at rest, produces carbon dioxide constantly. The amount is greatest immediately after excision.

2. The curve for the yield of carbon dioxide from frogs' muscle shows marked irregularities; but nearly always there is an increased yield at the third and at the sixth hour. After the seventh hour the yield becomes nearly constant until putrefaction sets in, when there is a sudden and enormous increase.

3. With mammalian muscle (cat and dog) the curve is much more regular. It falls rapidly for from three to six hours, then more gradually, but steadily for as long as ten hours.

4. Contracting muscles (frog), not fatigued, and lying free so as not to perform work, do not, as a rule, give off more carbon dioxide

than when at rest. If the muscle is made to work, the carbon dioxide is increased. This comparison has not yet been made with mammalian muscle.

5. There appears to be no difference in the yield of carbon dioxide from a muscle when stimulated through its nerve, and when stimulated directly.

6. A temperature between 18° and 30° C. does not appear to affect the yield of carbon dioxide.

7. Variations in the speed of the air current from 5 to 8 litres per hour do not affect the yield of carbon dioxide. When the current is shut off the absolute yield is greatly reduced.

8. Curare has no influence on the production of carbon dioxide in the unstimulated muscles of the frog.

9. Curarized frogs' muscles, when their nerves were stimulated, have not given constant results.

10. Curarized mammalian muscle (dog and cat), when their nerves are stimulated, gives off more carbon dioxide than when at rest. (This statement is based on only three experiments.)

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## THE RELATION OF TONUS CONTRACTION TO CONDUCTION IN SMOOTH MUSCLE.

BY W. T. PORTER, C. H. LAWRENCE, JR., AND L. H. NEWBURGH.

ABOUT 10 cm. of the small intestine was removed between double ligatures and placed in Ringer's solution (sodium chloride 0.9, calcium chloride 0.26, potassium chloride 0.04 per cent), in a glass dish over an incandescent lamp which maintained the solution at 38°C. The intestine was fixed at either end by an electrode attached to the side of the glass dish. A peristaltic wave was induced by cathodal stimulation with a constant current (four Daniell cells). The cathode was placed at the upper end of the intestinal segment. The constant current was shut off the moment the contraction began. A light pin attached to the base of a cork wedge resting on the intestine, transmitted to a light recording lever the moment at which the contraction wave passed beneath the wedge.

The moment of stimulation was recorded by an electro-magnetic signal in the galvanic circuit. The interval between the stimulation

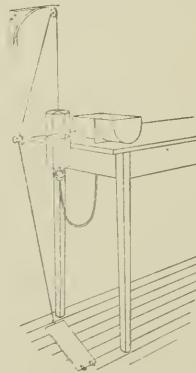
and the passage of the contraction over the cross section beneath the recording lever was measured before, during, and after the production of a tonus ring about 2 mm. in width. This tonus ring was produced by stimulation with from 4 to 15 maximal make and break induction currents at intervals of 1 second. The tonus ring remained markedly evident from one to two minutes after the induced current was shut off.

It was found that a ring of intestinal muscle in the middle of a piece of extirpated but surviving intestine can easily be thrown into a tonic contraction which far outlasts the stimulus. A peristaltic wave passing down the intestinal tube is delayed or wholly arrested on reaching the tonus ring. Indeed, the peristaltic wave often turns at the tonus ring and moves by antiperistalsis back to the end of the tube.

#### A HANDY VARNISHING APPARATUS.

By E. P. LYON.

THIS apparatus has been used in the physiological laboratory of St. Louis University for two years and is "student proof." The treadle is hinged to the floor. Stepping on the treadle raises the bottle and



VARNISHING APPARATUS.

fills the tray. Removing the foot allows the bottle to fall. A knot prevents its going too far. The varnish immediately flows back into the bottle. Dr. Koch tells me the same plan is used in the University of Missouri.

The following communications were read :

- GALVANOTROPISM OF VOLVOX. By O. P. TERRY (by invitation). This journal, 1906, xv, pp. 235-243.
- ON THE OCCURRENCE OF CHLORIDES IN THE NERVE AXON. By A. B. MACALLUM.
- ON THE OCCURRENCE OF CHLORIDES IN ANIMAL AND VEGETABLE CELLS. By A. B. MACALLUM.
- ON THE CONDUCTION AND CO-ORDINATION IN THE HEART. By A. J. CARLSON. This journal, 1906, xv, pp. 99-120.
- OSMOTIC PRESSURE AND HEART ACTIVITY. By A. J. CARLSON. This journal, 1906, xv, no. iv.
- SUPRARENAL TRANSPLANTATION. By F. C. BUSCH.
- SLEEP OF INFANTS. By C. F. HODGE.
- THE PRODUCTION OF GASTRO-INTESTINAL PERISTALSIS BY ERGOT AND ITS INHIBITION BY MAGNESIUM SULPHATE. By S. J. MELTZER and J. AUER.
- SOME OF THE CHEMICAL PHENOMENA OF MUSCLE FATIGUE. By F. S. LEE.
- THE OXIDATION OF SUGAR BY COPPER ACETATE. By A. P. MATHEWS and H. MCGUIGAN.
- PHARMACOLOGICAL ACTION OF METALS AT A DISTANCE. By A. P. MATHEWS.
- THE ACTIVITY OF THE HEART GANGLION IN THE ABSENCE OF OXYGEN. By H. H. NEWMAN (by invitation).
- ANTIBODIES PRODUCED BY THE INJECTION OF NUCLEO-PROTEIDS. By S. P. BEEBE.

The following communications were read by title :

- THE RESUSCITATION OF THE CENTRAL NERVOUS SYSTEM. By G. N. STEWART.
- THE HEMISECTION OF THE SPINAL CORD. By W. MILLS.
- FURTHER INVESTIGATIONS ON THE EFFECT OF PARTIAL STARVATION ON THE CENTRAL NERVOUS SYSTEM OF THE WHITE RAT. By S. HATAI (by invitation).
- THE COMPARATIVE CHEMICAL COMPOSITION OF THE HAIR OF DIFFERENT RACES. By P. B. HAWK and T. A. RUTHERFORD.

The following demonstrations were given :

- DEMONSTRATION OF HEART-BLOCK IN THE DOG. By J. ERLANGER.
- A NEW APPARATUS FOR THE QUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF CARBON DIOXIDE. By G. T. KEMP, O. O. STANLEY, and E. R. HAYHURST.
- DEMONSTRATION OF THE EFFECT OF EXCITATION OF THE VAGUS NERVE ON THE PULSE CURVE OBTAINED FROM THE LONGITUDINAL EXPANSION OF THE CAROTID. By W. P. LOMBARD.
- PHENOMENA OF SHOCK. By T. HENDERSON.

A METHOD OF STUDYING THE ACTION OF THE MUSCLES OF THE HIND LEG OF THE FROG. W. P. LOMBARD (for Mr. ABBOTT).

A METHOD OF STUDYING THE ACTION OF ADRENALIN, ETC., ON THE BLOOD-VESSELS OF THE ISOLATED BRAIN. By W. P. LOMBARD (for C. J. WIGGERS).

AN EXHIBITION OF APPARATUS. By W. P. LOMBARD.

AN IMPROVED APPARATUS FOR RECORDING THE SECRETION OF URINE. By E. M. HOUGHTON.

MODELS OF THE KIDNEY TUBULES. By G. C. HUBER.

A DEMONSTRATION OF APPARATUS. By L. FISHER (by invitation).

A DEMONSTRATION OF APPARATUS. By L. B. MENDEL.











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